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THE INTRAPERITONEAL ADMINISTRATION OF VIOSTEROL IN MICE

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The aim of this study was to determine if irradiated ergosterol[2] is efficacious when injected into the peritoneal cavity.

During the course of experiments dealing with the calcification of the Twort mouse carcinoma (in vivo) by means of viosterol (7), the animals receiving medication frequently had diarrhea. It was presumed that such an abnormal condition of the gastro-intestinal tract might have not only an adverse effect upon the health of the mice but might well lead to a non-utilization of much of the viosterol. Therefore, the present investigators were confronted with the finding of a parenteral route by which the activated ergosterol might act efficiently and yet cause no effects detrimental to the object of the investigation. The decision to inject the substance into the peritoneal cavity was influenced by the report of Koehne and Mendel (1) which stated that the Vitamin D principle in cod liver oil afforded some protection to rats when given in this manner, and our enthusiasm was kindled somewhat by the fact that (at that time) we found no articles dealing with this mode of administration for viosterol.

EXPERIMENTAL MATERIALS AND METHODS. The irradiated ergosterol, as received from the manufacturers, was concentrated in sesame oil to a biologically-assayed strength of 10,000 times the antirachitic potency of cod liver oil (10,000 CLO). In order to obtain solutions of 1,000 CLO and 2,000 CLO, which were used in certain phases of the present investigation,

[1] Submitted to the Faculty of the Yale School of Medicine as partial requirement for the degree of Doctor of Medicine.

[2] The viosterol was obtained through the courtesy of Mead Johnson & Co., Evansville, Indiana.

Such terms as viosterol, irradiated ergosterol, and activated ergosterol are used interchangeably throughout the present article.

proper dilutions of the 10,000 CLO preparation were secured by the addition of sesame oil, which was supplied by the manufacturers for this purpose.

The mice were adults of both sexes and in some instances were the same as those used for the above mentioned experiment concerning the calcification of a mouse tumor. The diet consisted of oats and lettuce. In the first experiment milk was given, but it was omitted in the later ones. All had free access to tap water. Only apparently healthy animals were introduced into the investigation, and careful attention was given to ventilation, light, and the weekly sterilization of cages. Despite these precautions, the colony was depleted several times by epidemics of mouse typhoid. However, this condition was finally overcome, and the data from affected animals were discarded.

Reasonable cleanliness was observed, but it was not found necessary to disinfect the skin or even to sterilize repeatedly the needle and syringe. Neither before nor at the time of necropsy was an infection ever noted. The sesame oil, containing the irradiated ergosterol, was slightly warmed in order to secure a uniform solution.

With the back of the mouse grasped in the usual manner and the abdomen turned uppermost, the desired amount of sesame oil was injected into the peritoneal cavity. The procedure proved to be extremely simple and reliable as determined by immediate and subsequent celiotomies. One person easily accomplished it, and many mice were administered to in a short time.

The silver nitrate method of Von Kossa was employed for the histologic identification of calcium. The counter stain was alum carmine. In certain instances hematoxylin and eosin were resorted to for the study of tissue structure. Seventy per cent ethyl alcohol was quite satisfactory as a fixing agent and was more convenient than formalin on account of having to keep the latter neutralized in order to prevent loss of calcium from the tissues due to an acid medium.

The daily, general condition of the animals was noted. Some were allowed to die. Others were killed when it became apparent that they would survive only a short time. Thus we had at our disposal tissues which were fresh and also those which had been subject to postmortem autolysis for a few hours. As would be expected, in the former instance the results were ordinarily more satisfactory as regarded the detection of calcium as well as the preservation of the normal histology.

GENERAL PLAN OF THE EXPERIMENTS. After preliminary investigations had been carried out, further studies were conducted, of which the two reported below may serve as examples.

In one of these inquiries, designated as experiment 1, a total of 27 mice was employed. Eleven of the 27 were given viosterol of 1,000 CLO (the

original 10,000 CLO having been diluted with sesame oil) over a period varying in the individual animal from 25 to 61 days. In the same experiment a second group, consisting of 9 mice, received equivalent amounts of sesame oil (which contained no activated ergosterol) during a time interval of 22 to 61 days. The third and final lot of experiment 1 contained 7 individuals, which had nothing but the regular diet and were sacrificed at intervals varying from 26 to 61 days.

In experiment 1 a total of 17 intraperitoneal injections of a sesame oil solution of viosterol (1,000 CLO) was given. The first 12 doses each contained 0.1 cc., which was increased to 0.133 cc. in each of the last 5 administrations. In each of the animals which survived the period of 61 days, the entire amount received by any one individual was 1.865 cc., and the average dose was 0.11 cc.

In experiment 2 a total of 35 mice was utilized. Twenty-four of these were given sesame oil solutions of viosterol (2,000 CLO and 10,000 CLO) over a period varying in the individual animal from 43 to 52 days. A second group in experiment 2 contained 11 mice, the members of which had only the regular diet and were sacrificed in from 43 to 51 days.

In the first 10 injections of experiment 2, a sesame oil solution of viosterol (2,000 CLO) was employed. In each mouse the dose of the oil varied from 0.048 cc. to 0.160 cc. with an average of 0.0832 cc. and a corresponding total of 0.832 cc. In the same experiment (no. 2), the original undiluted sesame oil solution of viosterol (10,000 CLO) was utilized in the last 9 injections, in this instance the amount varying in each mouse from 0.016 cc. to 0.128 cc. with an average of 0.094 cc. and a total of 0.846 cc. When the above values from the two divisions of experiment 2 are translated into terms of 1,000 CLO, the total dose received by each mouse in experiment 2 would have been equal in antirachitic potency to 10.124 cc. of a sesame oil solution of viosterol as used in experiment 1, which, in the same terms, would amount to an average injection of approximately 0.533 cc. for each of the 19 doses administered in experiment 2. Thus it will be seen that the mice in experiment 2 were given much greater relative amounts of activated ergosterol than those in experiment 1.

In order to arrive at a correct evaluation of the results produced by the viosterol, it was endeavored to control any effects which might have been caused by the sesame oil (p. 529, expt. 1, group 2).

Results. After about 30 days, depending somewhat upon the dosage employed, the mice exhibited anorexia followed by loss of weight. Continuation of the activated ergosterol resulted in bodily inactivity and ruffling of the hair. However, up to this point the cessation of viosterol administration was followed by apparent complete recovery of the animals. In the last stages of the illness there appeared extreme unsteadiness of movements, and the tails were cyanotic. From this point, even if medica-

tion were discontinued, the disease progressed steadily and rapidly until death.

In each mouse, which lived more than 30 days after receiving viosterol, abnormal calcium deposits were seen in various organs as revealed by the Von Kossa histologic method. This may be expressed in figures by stating

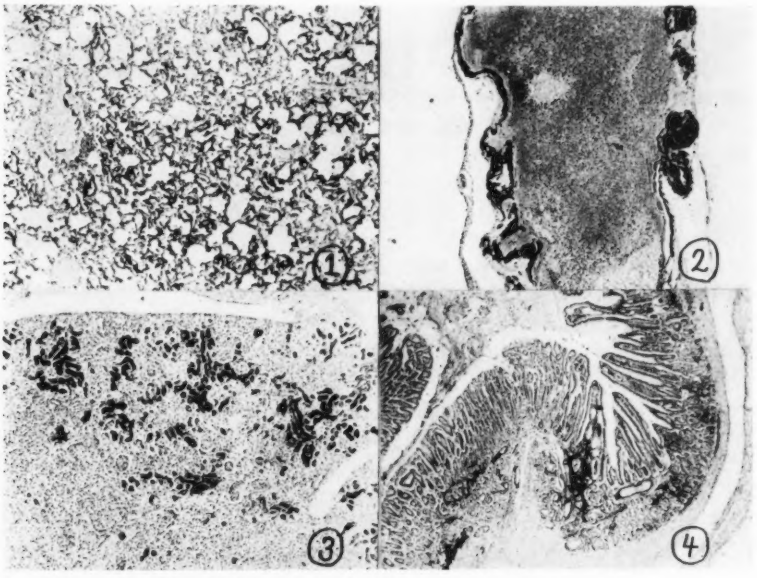

Fig. 1. ×45—Lung. Von Kossa stain. Calcium appears as black areas in the alveolar septa and in a large arteriole (left border of photograph).

Fig. 2. ×45—Aorta. Von Kossa stain. The black portions of the intima and media represent large calcium deposits.

Fig. 3. ×45—Kidney. Von Kossa stain. Many tubules are completely replaced by calcium, which shows as very black spots. Involvement of blood vessels is not seen in this part of the section but was noted in other portions. A partially calcified glomerulus may be observed near the left border of the photogram.

Fig. 4. ×25—Stomach and duodenum. Von Kossa stain. The black areas represent calcium. The pyloric muscle (projection in lower center) is seen separating the stomach on the left from the duodenum on the right. Calcification is evident in both organs.

that out of 35 animals which received activated ergosterol, there were 29 that exhibited pronounced calcification of certain viscera (figs. 1, 2, 3, and 4). The remaining 6 mice presumably lived too short a time (less than 30 days) for the lesions to become demonstrable.

We noted no calcific processes in the vena cava, esophagus, liver, pan-

creas, adrenal gland, ovary, testis, or cecum. In the *heart* and *spleen* the calcium deposits were limited to the arteries. In the *lung* calcium was found in the bronchial cartilages and mucosa, alveolar walls, and arteries. In the *aorta* very extensive calcification was seen in the intima and adjacent portion of the media. Calcific changes in the aorta appeared less often than in the stomach, lungs and kidney; but, when present, they were very marked. In the *kidney* calcium was deposited within the cells and the lumina of convoluted tubules, the intertubular spaces, arteries, and infrequently in the mucosa of the pelvis. In the *stomach* the cells of the glands, the muscularis mucosa, and the arteries of the submucosa were constantly and markedly affected. Pronounced calcification was occasionally seen in the prepyloric portion of the stomach, a finding contrary to that of another observer (3).

In no instance did the sesame oil per se have any apparent influence upon the deposition of calcium in the various organs.

Discussion. It may be seen that in the main we have confirmed the observations of other workers as to the deposition of calcium by means of viosterol. In so doing, *the efficacy of the intraperitoneal route of administration has been established.*

However, we differ from Kreitmair and Moll (2) in that we did not find deposits of calcium in the muscles and adrenals, and from Smith and Elvove (5) in that we observed calcification of the pulmonary and renal arterioles, thus in the case of the kidney supporting the findings of Spies and Glover (6), and differing from Laas (3) in noting calcific changes in the prepyloric portion of the stomach and in the duodenum. It is quite possible that certain differences as observed by various workers may have resulted from diversity in the susceptibility of animals and in the duration of therapeusis, or in the amount and potency of the viosterol employed, as well as the routes and methods of administration.

A doubt as to the efficacy of the intraperitoneal injections of viosterol might arise by assuming that at times the needle entered the gut, thereby depositing the substance within the gastro-intestinal tract, and that the results which we secured may have been obtained by absorption through this route rather than by way of the peritoneum. It is not likely that such accidents could have happened sufficiently often to have seriously influenced our results. It will be remembered that our vehicle for the irradiated ergosterol was sesame oil. In no instance did we ever find the latter within the intestines, and we were always able to demonstrate it in the peritoneal cavity soon after injection and also after a lapse of several days. Furthermore, no infection was noticed, a thing which might have happened by even a small puncture of the gut.

It would appear, therefore, that the injection of activated ergosterol into the peritoneal cavities of mice has proven to be easy, reliable, and safe.

As this work was drawing to a close, Reed and Thacker (4) reported that viosterol was well utilized by dogs in whom it had been injected into the peritoneal cavity. Thus our experience in mice confirms theirs in dogs. However, they stated that when once the toxic symptoms of overdosage occurred they progressed, and death inevitably resulted. Often when our animals evidenced profound toxicity, as indicated by ruffled hair, anorexia, and disinclination to activity, we were able to restore them to normalcy by omitting the medication for a time. This difference as to recovery may have been due to proportionate variations in single dosages; for, when once the substance was injected into the peritoneal cavity, it was not removed, and a large amount of the sesame oil might have contained sufficient viosterol for the prolongation of toxic effects, finally terminating in the death of the animal whether more injections were made or not. This view is supported by the statement of Smith and Elvove (5) to the effect that the toxicity of activated ergosterol was related to the size of the single dose rather than to the total amount given over a certain period.

Reed and Thacker (4) reported calcification in the kidneys of their dogs, but they did not describe the technic employed for the histologic identification, and they apparently placed no emphasis upon the finding.

Since vitamin D is absorbed via the peritoneum, it may be that other fat soluble substances are capable of like action. Koehne and Mendel (1) have shown that this is true to a certain extent for vitamin A.

We have made no attempt to investigate the effects of the sesame oil and cannot therefore give any data concerning it except that it was apparently absorbed to a partial degree and did not cause obvious consequences in the animals.

It is our belief that the intraperitoneal method is applicable for laboratory animals, where the utilization of an exact amount of viosterol is desirable and where the avoidance of diarrhea is advisable.

Also, we wish to advocate the use of this technic, or a modification, in studies concerned with the way in which vitamin D acts, for it is now thought by many that its function is to increase the absorption of calcium from the intestinal tract. If this be true, then vitamin D which has been introduced parenterally, must be excreted into the gut or else be able to elaborate changes in the intestinal epithelium either by its actual presence there or by the action of secondarily evolved substances.

SUMMARY. The purpose of this study was to determine if viosterol could act efficaciously when injected into the peritoneal cavity of mice. The problem arose in connection with another which required the utilization of large, definite amounts of irradiated ergosterol (7) and the avoidance of diarrhea, which in our experience frequently accompanied the gastric and stomatic routes of administration.

A simple, safe, and reliable method has been described for the intraperitoneal administration of viosterol in mice.

The general condition of the animals and the histologic identification of calcium were the criteria employed for the evaluation of effects.

Two experiments have been reported, comprising a total of 62 mice, 35 of which were given viosterol intraperitoneally. In addition, 18 normal animals received nothing but the routine laboratory diet. Nine other mice were run as controls for any effects that might be produced per se by the sesame oil, since this was used as a vehicle for the viosterol.

The animals which lived more than 30 days (29 mice out of a total of 35) after the beginning of the viosterol injections revealed calcification in various organs, being most marked in the lung, aorta, kidneys, and stomach. These findings have been briefly compared with those of workers using other modes of administration, and the marked similarity of results indicates that the action of viosterol by way of the peritoneum is the same as by other routes in so far as the general condition of the animals and the deposition of calcium are concerned.

The possibility of the needle occasionally piercing the gut has been discussed with the conclusion that such was unlikely, and that infrequent mishaps of that nature could not have accounted for the marked effects which were uniformly obtained.

In the discussion we have pointed out certain applications that might be made of parenteral routes for the administration of fats and oil, including substances solvent in them.

CONCLUSIONS

1. The intraperitoneal injection of viosterol in mice has been established as a safe, simple, and reliable method of administration.

2. Calcium deposits were most marked in the lungs, stomach, kidney, and aorta.

3. The sesame oil (vehicle for the viosterol) did not have any demonstrable influence upon the calcific processes.

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THE EXCRETION OF URINE IN THE DOG

VI. The Filtration and Secretion of Exogenous Creatinine

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The excretion of ingested creatinine was recommended by Rehberg (1926) as a measure of glomerular filtration on the grounds that this substance is concentrated by the kidney to a greater extent than any other substance in the urine. Behre and Benedict (1922) had previously expressed doubts about the existence of creatinine in normal blood in sufficient quantities to account for the creatinine normally excreted, but Rehberg concluded that, since ingested creatinine is excreted by the kidney with the same readiness as the chromogenic substance or substances which are normally present in the blood, the main part of this chromogenic substance must be really creatinine.

Behre and Benedict's conclusion that part of the chromogenic substance in the blood is not creatinine has been confirmed by Gaebler and Keltch (1928), by Gaebler (1930) and by experiments of ours reported in this paper. Meanwhile Marshall and Grafflin (1928, 1930, 1932) have shown that exogenous creatinine is secreted by the aglomerular fish kidney, and also by the glomerular fish kidney both when the glomeruli are functional and when they have been rendered non-functional by the irritant action of phlorizin; and Clark and Smith (1932) have shown that exogenous creatinine is secreted by the elasmobranch kidney, although it is now well established that this substance is not normally present in significant amounts in the urine of these or other fishes.

These facts render the use of creatinine as a measure of glomerular filtration in the mammal suspect, and it is of interest, therefore, to compare the excretion of creatinine with the excretion of the non-metabolized sugars which Jolliffe, Shannon and Smith (1932) have recommended for this purpose.

The sugar methods used here have been fully described in the paper referred to above, and only a brief comment will be added here. For the determination of creatinine at low plasma levels, we have endeavored to devise a method which will distinguish true creatinine from other chromogenic substances present in plasma and urine. This method is described

in an appendix to this paper. By this method at least three substances, or groups of substances, can be distinguished in plasma:

1. Creatinine (of either exogenous or endogenous origin; in this category there will also fall the substituted creatinines which behave toward Lloyd's reagent like creatinine).[1]

2. Non-creatinine chromogenic substances which are distinguishable from creatinine by the fact that they are not absorbed by Lloyd's reagent.

3. A non-chromogenic substance (other than creatinine or creatine) which yields creatinine by absorption and liberation from Lloyd's reagent. For further information on this substance the reader is referred to the original observations of Gaebler.

We have never found more than 0.5 mgm. per cent of non-creatinine chromogenic substance in the plasma of dogs fed upon our cracker meal diet or upon meat, and consequently when the plasma creatinine level is raised to 10 mgm. per cent or higher, these substances are not a serious source of error in the determination of the creatinine concentration by Folin (1919) method. The concentration of non-creatinine chromogenic substances in the relatively dilute urines which we have analyzed has not exceeded 1.0 mgm. per cent, which amount is, of course, so small that it also can be neglected in experiments in which large quantities of creatinine are administered. The excretion of non-creatinine chromogenic substances and the question of the origin of the creatinine present in normal urine, will be referred to in the latter part of this paper.

The secretion of exogenous creatinine. The first experiments which we wish to describe were made with dog 36; we have used this dog in many previous experiments and consequently have numerous data on her relative to the excretion of urea, xylose and sucrose under various conditions. The first three experiments (expts. 140, 141 and 143) concern the excretion of xylose and creatinine while the dog was on a maintenance diet of cracker meal, butter and sugar, and when the glomerular (xylose) clearance was at a minimum. In these experiments varying quantities of creatinine were administered subcutaneously, along with constant quantities of xylose by stomach. For the detailed data the reader is referred to table 1 and its accompanying protocols. The results of these experiments show clearly that the rate of creatinine excretion relative to the plasma level of creatinine—i.e., the creatinine clearance—is greater by about 40

[1] We have observed that methylcreatinine, benzylcreatinine and benzoylcreatinine (the latter prepared either from benzoylchloride or benzoic anhydride) are absorbed by Lloyd's reagent. It is obvious, therefore, that such substances would behave like creatinine in any method depending upon the use of this absorbent and probably also in any method based upon the conversion of creatinine to creatine. We are indebted to Dr. Isador Greenwald for the substituted creatinines used in making the above tests.

TABLE 1

A comparison of xylose and creatinine clearances at varying plasma levels of creatinine

PERIOD	TOTAL CONCURRENT TIME	URINE FLOW PER MINUTE	XYLOSE		CREATININE		CM = UV/P / S.A.		CM CREATININE / CM XYLOSE	CM UREA / CM XYLOSE
			Plasma	Urine	Plasma	Urine	Xylose	Creatinine		
			Expt. 140. Dog 36							
	minutes	*cc.*	*mgm. per 100 cc.*	*mgm. per 100 cc.*	*mgm. per 100 cc.*	*mgm. per 100 cc.*				
1	20	8.50	170.5	868	55.9	422	60.1	88.9	1.48	
2	40	5.20	173.5	1,225	52.7	527	51.0	72.2	1.42	
3	60	5.75	175.5	1,164	45.7	427	53.0	74.6	1.41	
4	81	5.47	176.5	1,234	43.0	406	53.1	71.7	1.35	
								Average:	(1.41)	
			Expt. 141. Dog. 36							
1	20	6.50	168.5	1,150	42.5	395	61.6	83.9	1.36	
2	42	5.10	174.4	1,477	39.2	473	60.0	85.5	1.42	
3	60	3.06	159.6	2,010	34.2	593	53.5	73.7	1.38	
4	85	0.60*	135.2	2,900	28.8	863	17.9*	25.0*	1.40	
								Average:	(1.39)	
			Expt. 143. Dog 36							
1	21	3.33	92.2	1,100	31.2	544	55.7	80.6	1.45	0.687
2	41	4.05	98.9	926	27.2	376	52.7	77.8	1.48	0.700
3	62	4.95	103.0	921	23.2	283	61.5	83.8	1.36	0.657
4	82	5.10	105.2	930	20.7	251	62.6	85.9	1.37	0.645
								Average:	(1.42)	
			Expt. 159. Dog 36							
			In this experiment "true" creatinine values for plasma and urine are given							
1	31	3.74	125.0	1,604	25.10	406	66.7	84.1	1.26	
2	60	5.07	135.8	1,357	22.50	297	70.2	92.9	1.32	
3	92	5.72	134.0	1,299	18.60	244	77.0	104.2	1.35	
4	121	3.40	109.8	1,900	16.40	383	81.7	110.4	1.35	
5	150	1.62	83.4	2,750	14.50	670	74.2	103.9	1.40	
6	179	1.14	59.7	2,780	12.00	803	73.7	105.8	1.44	
	(322)	** See note A: more xylose administered								
7	346	5.70	169.5	1,579	5.56	76	73.7	108.2	1.47	
8	376	3.77	175.5	2,350	5.14	90	70.1	91.7	1.35	
9	410	3.18	133.3	2,372	4.35	107	78.5	108.7		
10	436	1.62	98.1	3,150	3.85	173	72.3	101.2	1.40	
11	466	1.14	75.5	3,300	3.50	222	69.3	99.8	1.44	
12	496	0.87	52.6	3,040	3.00	253	69.7	101.9	1.46	
								Average:	(1.39)	

* Urine spilled.

** Urine from washout period discarded.

to 50 per cent than is the rate of xylose excretion relative to the plasma level of xylose. (Our figures are corrected to the plasma clearance per square meter of body-surface area, as in previous papers, to facilitate the comparison of various dogs.)

On the basis of the evidence, presented by Jolliffe, Shannon and Smith (1932), that the rate of excretion of xylose relative to the plasma concentration measures within a few per cent the quantity of glomerular filtrate, or the glomerular clearance, the results of experiments 140, 141 and 143 must be accepted as evidence that a considerable quantity of creatinine is removed from the blood by some means other than, and in addition to that which is cleared by glomerular filtration. We infer that this additional quantity of creatinine represents a tubular excretion of this substance from the post-glomerular plasma, and we will therefore refer to it as the *tubular clearance*, in contradistinction to the *glomerular clearance*. Thus, in period I of experiment 140, the glomerular clearance of creatinine (theoretically the same as the glomerular clearance of xylose) is 60.1 cc. per square meter per minute, and the tubular clearance is 28.8 cc. per square meter per minute (88.9–60.1); we may conveniently identify the relative values of these by saying that the tubular clearance is 48 per cent of the glomerular clearance.

The difference between the xylose clearance and the creatinine clearance cannot be due to the excretion of what we may designate the normal or endogenous creatinine because the normal excretion of creatinine for a dog of this size is not greater than 0.3 mgm. per minute, whereas the average discrepancy between the xylose clearance and the creatinine clearance in experiment 140 is 22.3 cc. of plasma per minute containing 49.3 mgm. per cent or 11 mgm.,—i.e., 33 times the normal excretion. Instead, we are led to conclude that preformed creatinine is removed from post-glomerular blood by tubular activity and secreted directly or indirectly into the tubular urine.

The first question which arises in connection with the tubular secretion of creatinine is its relationship to the concentration of this substance in the plasma. In experiments 140, 141 and 143 the plasma level varied from 55.9 down to 20.7 mgm. per cent. There are irregularities in the relative tubular clearance from 37 to 48 per cent of the glomerular clearance, but the variations are distributed irregularly and are probably due to experimental error; if averages are taken for each experiment, as is indicated in table 1, the relative tubular clearance for the three experiments at average plasma levels of 49.3, 36.2 and 25.6 respectively, was 41, 39 and 42 per cent. In experiment 159, performed on this same dog two and a half months after experiment 143, the plasma level ranged from 25.0 down to 3.0 mgm. per cent; in this experiment the tubular clearance was 38 per cent of the glomerular clearance. (In expt. 159 the true creatinine, as

determined by the method described in the Appendix, is given for the plasma while the urine concentration in this experiment and the plasma and urine concentrations in expts. 140, 141, and 143 are for the total chromogenic substance.) These four experiments indicate then, that at plasma levels above 3.0 mgm. per cent the tubular excretion of creatinine is a linear function of the plasma concentration and that the tubular clearance is therefore independent of the plasma concentration. Since the glomerular excretion of creatinine must be assumed to be a linear function of the plasma concentration so long as the rate of glomerular filtration is constant, it follows that, all other conditions being equal, the total excretion of creatinine will likewise bear a linear relationship to the plasma concentration. MacKay and Cockrill (1930) have demonstrated that such a linear relationship exists in the rabbit under "standard conditions," but they have erroneously argued that this fact may be taken as evidence that creatinine is excreted in this animal solely by glomerular filtration. On the contrary, it is quite possible that the tubular excretion is proportional to the plasma concentration in the rabbit as in the dog, in which case the total excretion would bear the same relationship.

The influence of diet upon the tubular excretion of creatinine. It is held on good grounds that the blood to the renal tubules in the mammal is supplied entirely from the efferent glomerular arterioles. Except for such changes in glomerular filtration as might result from changes in local glomerular pressure it would be expected, therefore, that the glomerular clearance and the tubular clearance would be affected in the same direction by changes in number of active glomeruli and in blood flow through the kidney, and where the tubular excretion is a linear function of the plasma concentration, as in the case of creatinine, these should be affected to approximately the same extent.

It has been shown in a previous paper that the rate of glomerular filtration (and the urea clearance) can vary in a single dog as much as three and a half fold in relation to the dietary regime (Shannon, Jolliffe and Smith, 1932) and it is known that the creatinine clearance increases and decreases under these same conditions (Jolliffe and Smith, 1931). It therefore seemed important to examine the effects of diet on the tubular clearance of creatinine relative to the glomerular clearance as measured by xylose.

With this point in mind we transferred dog 36 from the cracker meal diet which had been used throughout the above experiments, and upon which the glomerular clearance was low (50 to 60), to a mixed diet. In the next two experiments (expts. 147 and 149, table 2) after seven days on the mixed diet, and after eating a meat meal, the glomerular clearance ranged from 90 to 97. Thus by dietary means the glomerular clearance was nearly doubled, but the proportion between the glomerular clearance and the tubular clearance of creatinine remained fairly constant, the latter

TABLE 2

Effect of diet and erythroltetranitrate on xylose and creatinine clearances

PERIOD	TOTAL CONCURRENT TIME	URINE FLOW PER MINUTE	XYLOSE		CREATININE		CM = UV/P / S.A.		$\frac{CM\ CREATININE}{CM\ XYLOSE}$	$\frac{CM\ UREA}{CM\ XYLOSE}$
			Plasma	Urine	Plasma	Urine	Xylose	Creatinine		
			Expt. 147. Dog 36							
	minutes	*cc.*	*mgm. per 100 cc.*	*mgm. per 100 cc.*	*mgm. per 100 cc.*	*mgm. per 100 cc.*				
1	30	1.90	95	3,255	30.9	1,404	90.4	119.7	1.32	0.790
2	60	1.70	89	3,645	27.4	1,446	96.7	124.8	1.29	0.763
3	87	1.59	79	3,470	24.7	1,417	97.0	126.5	1.30	0.796
								Average:	(1.30)	
			Expt. 149. Dog 36							
1	31	2.45	146	3,895	35.6	1,290	90.8	123.0	1.36	0.807
2	61	2.20	130	4,065	28.8	1,244	95.5	131.8	1.38	0.777
3	91	1.66	100	4,085	24.9	1,333	94.2	123.5	1.31	0.764
								Average:	(1.35)	
			Expt. 158. Dog 36							
1	22	2.68	121	3,285	27.4	985	101.0	133.8	1.33	
2	44	2.77	127	3,390	27.6	990	102.7	138.0	1.34	
3	65	3.23	134	3,020	27.0	790	101.2	131.3	1.30	
	See note A: E. T. N. administered							Average:	(1.32)	
4	95	4.23	136	2,000	28.4	543	86.4	112.5	1.30	
5	117	2.27	134	3,450	26.5	896	81.2	106.8	1.31	
6	151	1.82	102	3,470	22.5	995	86.1	111.7	1.30	
7	181	1.53	74	3,110	18.5	1,015	89.3	116.5	1.30	
8	215	1.09	56	3,300	14.7	1,143	89.2	117.7	1.31	
								Average:	(1.31)	
			Expt. 157. Dog 43							
1	15	2.27	199	3,290	82.0	1,547	40.8	46.6	1.14	
2	35	2.40	206	3,140	75.5	1,345	39.7	46.5	1.16	
3	56	2.38	209	3,305	70.2	1,245	40.9	45.9	1.12	
	(99)	** See note A: E. T. N. administered.						Average:	(1.14)	
4	127	3.46	246	3,740	60.0	1,111	57.2	69.6	1.21	
5	164	3.24	224	3,830	55.0	1,053	60.2	67.4	1.10	
6	201	2.62	200	4,070	51.0	1,178	58.0	65.8	1.13	
7	235	2.21	167	3,970	47.0	1,333	57.1	68.1	1.19	
8	265	1.94	133	4,630	41.9	1,646	73.4	82.8	1.12	
9	295	1.53	104	3,540	38.7	1,507	56.6	64.8	1.14	
								Average:	(1.15)	

** Urine from washout period discarded.

dropping only from an average of 40 per cent of the glomerular clearance on the cracker meal diet (expts. 140, 141, 143 and 159) to 33 per cent in the mixed-diet and post-prandial experiments (expts. 147 and 149). This result suggests that only a small part of the increased glomerular activity in the latter experiments is due to increased glomerular pressure, and that the greater part of the increase in glomerular activity is accompanied by an increase in blood flow to the tubules.

To examine this point farther, in the last experiment and while the dog was still on a mixed and meat diet, a vasodilator was administered after three control periods (expt. 158). The vasodilator was erythroltetranitrate which was selected because it has a slow and prolonged action. During the three control periods the glomerular clearance was 100; this dropped to 80.9 in the second period after the erythroltetranitrate was injected. Throughout the course of the experiment, however, the relative tubular clearance of creatinine remained remarkably constant at 30 to 33 per cent of the glomerular clearance.

In another experiment of this same type (expt. 157), but performed on another dog which was being fed the cracker meal diet, and which therefore had a low glomerular clearance, the erythroltetranitrate had a reverse effect; the glomerular clearance rose from 40 before the injection of the drug to a maximum of 73 afterward. It is of course impossible to interpret these results accurately in terms of immediate causes, since the drug probably produces opposing effects; while it would probably tend to increase the number of open or active glomeruli and hence the total filtering surface and blood flow, it would at the same time be expected to decrease the rate of glomerular filtration by lowering the local glomerular and the systemic arterial pressure. Whether the net rate of glomerular filtration would increase or decrease would then depend on the predominance of these opposing effects. But the relative constancy of the ratio of the glomerular to the creatinine clearance in the above experiments in which the glomerular clearance was nearly doubled by diet, and profoundly modified in diverse directions by a vasodilator drug, indicates that changes in the rate of glomerular filtration are for the most part accompanied by parallel changes in blood flow to the tubules.

(It may be noted that dog 43, used in this last experiment, showed a relatively lower creatinine clearance than did dog 36; under comparable conditions the tubular clearance of creatinine was 40 per cent of the glomerular clearance in dog 36, 15 to 30 per cent in dog 43 and 17 to 31 per cent in dog 30.)

The effect of phlorizin on the secretion of exogenous creatinine.[2] Clark

[2] Poulsson (1930) has compared the excretion of creatinine with glucose in phlorizinized dogs. With one exception, the U/P ratios for creatinine in his experiments were greater than for glucose, the glucose/creatinine ratios varying from 1.10

TABLE 3

Effect of phlorizin on secretion of creatinine, etc.

PERIOD	TOTAL CONCURRENT TIME	URINE FLOW PER MINUTE	XYLOSE		CREATININE		GLUCOSE		$CM = \frac{UV}{P} / S.A.$			CM CREATININE / CM XYLOSE	CM GLUCOSE / CM XYLOSE	CM Cl / CM XYLOSE
			Plasma	Urine	Plasma	Urine	Plasma	Urine	Xylose	Creatinine	Glucose			
									Expt. 155. Dog 30					
	minutes	*cc.*	*mgm. per cent*	*mgm. per cent*	*mgm. per cent*	*mgm. per cent*	*mgm. per cent*	*mgm. per cent*						
1	16	2.00	151	3,595	87.8	2,438			55.3	64.6		1.17		0.051
2	35	3.10	161	3,540	89.2	2,397			53.7	65.6		1.22		0.037
3	63	2.00	165	3,880	89.1	2,420			54.7	63.2		1.16		0.019
	See note A: phlorizin administered								Average:			(1.19)		
4	79	2.43	163	2,765	83.5	1,471			48.0	49.8		1.04		0.171
5	100	3.28	157	2,025	79.0	1,053			49.3	50.8		1.03		0.180
6	130	2.06	130	2,590	75.0	1,505	102	1,957	47.7	48.1	46.0	1.01	0.962	0.042
	See note B: more phlorizin administered								Average:			(1.02)		
7	150	1.90	116	2,313	70.0	1,385	102	1,988	44.1	43.7	43.1	0.99	0.978	0.081
8	170	2.05	104	2,010	65.5	1,238	103	1,860	46.1	45.1	43.1	0.98	0.935	0.086
9	190	1.95	100	2,090	63.5	1,415	104	2,312	47.4	50.5	50.4	1.06	1.06	0.078
									Average:			(1.00)	(0.984)	
									Expt. 160. Dog 36					
														CM UREA / CM XYLOSE
1	20	3.50	186	2,272	24.4	412	77	0.0	59.4	82.0	0.0	1.38	0.0	0.752
2	49	7.62	192	3,000	22.0	457	78	0.0	56.8	75.5	0.0	1.33	0.0	0.756
3	76	2.73	177	2,886	19.5	390	76	0.0	61.8	75.8	0.0	1.23	0.0	0.726
	See Note A: phlorizin administered								Average:			(1.32)	(0.0)	0.743
4	106	2.63	145	2,150	17.5	273		1,158	54.2	56.9		1.05		0.736
5	135	2.34	115	2,069	15.6	278	89	1,574	58.4	57.9	59.4	0.99	1.02	0.672
6	162	2.14	87	1,800	13.9	288	75.6	1,535	61.6	61.6	60.3	1.00	0.98	0.673
									Average:			(1.01)	(1.00)	(0.693)

and Smith (1932) have found that, as judged by the simultaneous excretion of xylose, creatinine is abundantly secreted by the elasmobranch kid-

to 1.27 in one experiment, from 1.05 to 1.31 in a second, and from 0.91 to 1.20 in a third. Nevertheless he concluded that the correspondence between these ratios was such as to establish that creatinine is excreted exclusively by filtration.

Apart from our present demonstration that phlorizin abolishes the tubular excretion of creatinine, we believe that Poulsson's experiments are open to several criticisms; the plasma and urine sugars were determined by different analytical methods and the plasma sugar was determined by the Hagedorn-Jensen method in which phlorizin has

ney. This secretory activity was observed to be depressed by phlorizin, and in some instances it was completely abolished so that the total excretion of creatinine decreased until it corresponded to the quantity excreted

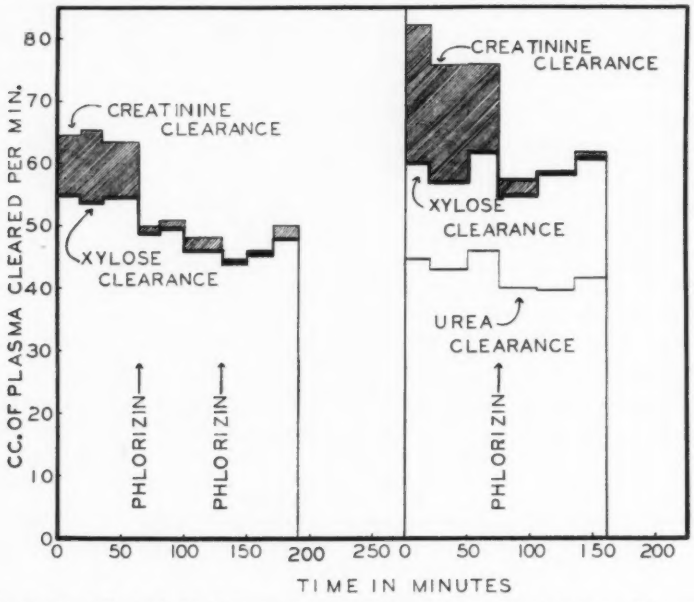

Fig. 1. The effect of phlorizin on the tubular secretion of creatinine. The creatinine clearance exceeds the xylose (glomerular) clearance in the normal dog by 15 to 50 per cent; when phlorizin is administered the tubular secretion of creatinine is arrested and consequently the creatinine clearance drops to the xylose or glomerular level. Phlorizin does not significantly affect the xylose or urea clearances, but it brings the glucose clearance up to the xylose clearance.

by glomerular filtration (this being the minimum, of course, if we assume that no creatinine is reabsorbed by the tubules).

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a strong reducing action (cf. Marshall, 1932); the urine sugars were determined by Benedict's titration method, in which phlorizin interferes with the end point. Since something over 200 mgm. per kgm. of phlorizin were given subcutaneously we may expect, if this substance is absorbed as quickly as sucrose, for example, to obtain from 10 to 30 mgm. per cent of phlorizin in the plasma and 20 to 50 times this concentration in the urine. In one experiment the non-glucose reducing substance in blood was determined by prolonged fermentation with yeast, but since this was not done on each plasma sample this precaution would not circumvent the difficulty. In addition a relatively enormous correction for the reducing power of creatinine, amounting to 50 per cent as its glucose equivalence, was necessary with the Hagedorn-Jensen method. These sources of error raise a serious doubt about the accuracy of the glucose U/P ratios observed by Poulsson.

It is of particular interest, therefore, to observe the effects of phlorizin on the excretion of creatinine in the dog. In experiments 155 and 160 (table 3) the normal xylose and creatinine clearances were observed in several control periods; phlorizin was then administered by intravenous and subcutaneous injection. (Our phlorizin is carefully purified by recrystallization from alcohol, as recommended by Lusk, 1928.) It will be seen from these experiments, which are also illustrated in figure 1, that phlorizin instantly and completely abolishes the extra excretion of creatinine, reducing the total creatinine clearance to the glomerular level. No explanation can be advanced for this action of phlorizin, but it may be reiterated, as Clark and Smith (1932) have pointed out, that a phlorizinized animal is hardly suitable for the examination of "normal" renal function.[3]

The action of phlorizin in arresting the tubular secretion of creatinine (as we interpret the excessive creatinine clearance observed in the normal animal) without significantly affecting the xylose or urea clearances is a further though indirect validation of our thesis that xylose measures the rate of glomerular filtration.[4]

[3] In view of the action of phlorizin in arresting the secretion of creatinine we have examined its effects on the excretion of a few other substances. In experiment 155 phlorizin did not significantly affect the excretion of Cl; the Cl clearance varied slightly immediately after the phlorizin was given, but it did not in any instance approach the glomerular clearance as would have been the case had this drug depressed the reabsorption of Cl to any considerable extent. In experiment 160 no creatine and only the faintest traces of phosphate (less than 0.1 mM per liter) were found in the urine either before or after phlorizin, so that, if these substances are filtered from the plasma, this drug does not arrest their reabsorption by the tubules. The excretion of water is obviously not affected by phlorizin except as the glucose constitutes an added osmotic factor in the urine (cf. expts. 155 and 160 in this paper, and expts. 87, 88, 92, 96 and 100 reported by Jolliffe, Shannon and Smith, 1932). Therefore the blocking of the reabsorption of glucose and of the secretion of creatinine remain the only two renal effects known to be exerted by this drug.

[4] Attention should be called again to the significance of the fact that phlorizin does not affect the excretion of xylose relative to urea (see the ratios of the xylose: urea clearances in expt. 160) as was pointed out by Jolliffe, Shannon and Smith (1932). This fact is substantial evidence that the effect of phlorizin consists of depressing the excretion of creatinine rather than increasing the excretion of xylose, since it is very unlikely that the drug would block a hypothetical reabsorption of urea and xylose to exactly the same extent. Moreover, the usual effect of phlorizin on the xylose clearance is not to increase it, as one would have to assume if the excretion of creatinine were taken as a measure of glomerular filtration, but rather to decrease the clearance as though there were some irritant action exerted upon the glomeruli. It is interesting to note in this connection that Marshall and Grafflin (1932) have found that relatively small doses of phlorizin cause the glomeruli in the sculpin to close up completely for several hours. No such marked result has been observed in the dog, although the administration of phlorizin is usually followed by a decrease in the xylose clearance. This effect may be due in part to hemolysis, since we have always given part of our phlorizin intravenously and since hemoglobin is known to produce a transient decrease in renal blood flow.

The excretion of endogenous creatinine. It is impossible by available methods to determine accurately the normal true creatinine in the plasma, and more particularly in urine, in animals to which xylose has been administered because of the interference of the sugar, as pointed out in the description of our creatinine method. Consequently it has not been possible for us to compare the excretion of xylose with the excretion of normal or endogenous creatinine. We have, however, made analyses on the plasma and urine of dogs which never had received injections of creatinine with the idea of determining whether or not the normal rate of excretion of creatinine falls within the same range as in dogs to which creatinine had been administered.

TABLE 4

The distribution of creatinine and non-creatinine chromogenic substance in normal plasma and urine

DOG	SURFACE AREA	SPECIMEN	(1) TOTAL CHROMOGENIC SUBSTANCE	(2) AFTER LLOYD'S	(3) CREATININE BY DIFFERENCE	(4) CREATININE BY RECOVERY	U/P	V	(5) $\frac{UV}{P}/SA.$	DIET
	sq. m.		*mgm. per 100 cc.*	*mgm. per 100 cc.*	*mgm. per 100 cc.*	*mgm. per 100 cc.*		*cc. per mm.*		
49	0.70	Plasma	0.84	0.17	0.67	0.67	32.6	1.02	47.5	Cracker meal
		Urine	22.70	0.83	21.87	21.50				
59	0.42	Plasma	0.77	0.15	0.62	0.65	21.7	1.19	61.6	Mixed
		Urine	14.00	0.52	13.48	12.90				
60	0.44	Plasma	0.53	0.17	0.36	0.52	57.2	0.64	83.3	Mixed
		Urine	21.30	0.68	20.62	25.00				

Data bearing on this point are given in table 4. In these analyses we have determined the total chromogenic substance in plasma and urine, using an aliquot of 2 cc. of the former and 1 cc. of the latter (column 1); the non-creatinine chromogenic substance remaining after extraction with Lloyd's reagent (column 2); true creatinine, as calculated by difference between (1) and (2); creatinine recovered from the Lloyd's reagent used in (2) by treatment with MgO (column 4).

Confirming Behre and Benedict (1922), Gaebler and Keltch (1928) and Gaebler (1930), we find a considerable moiety of the total chromogenic substance in plasma is not absorbed on Lloyd's and conclude that it is not creatinine. In other experiments we have found larger quantities of non-creatinine chromogenic substance than are given in table 4; for example, in dog 36, experiment 159, this moiety was 0.5 $\pm$0.05 mgm. per cent in all

plasma samples, and in another dog we observed 0.61 mgm. per cent. It is apparent that there may be sufficient non-creatinine chromogenic substance in plasma to introduce a serious error in creatinine clearance determinations based upon total plasma creatinine unless the plasma creatinine is 10 mgm. per cent or higher. It is interesting to observe that the non-creatinine chromogenic substance is not concentrated in the urine to the same extent as is creatinine (or even, we may suppose, as xylose); if this moiety is filtered through the glomeruli, it must be reabsorbed by the tubules.

Regarding the recovery of the absorbed creatinine from the Lloyd's reagent, it will be noted that in two instances (dogs 49 and 59) this recovery was almost perfect. In the third instance (dog 60) more creatinine was recovered from both the plasma and the urine than had been absorbed; it may be that this excessive recovery signifies the presence of the substance which Gaebler has described, and which is converted to creatinine by treatment with Lloyd's.

When the creatinine clearance is calculated from the true creatinine in the plasma and urine (column 3) of the above experiments, values are obtained (see column 5, table 4) which lie well within the range of the creatinine clearances which have been reported previously by Jolliffe and Smith (1931) and in this paper (i.e., between 40 and 150 per sq. m.). Behre and Benedict (1922) suggested, when they ascertained that not all the chromogenic substance in the blood is creatinine, that there might not be enough creatinine in the blood to account for the normal excretion of this substance. The above observations indicate, on the contrary, that it may not be necessary to infer the renal formation of creatinine from some precursor in order to account for the normal excretion of this substance. We make this point tentatively, however, because the experimental method is none too good, and at best it is quite non-specific since it includes substituted creatinines (methyl-creatinine, etc.) in the moiety which we have called creatinine.

SUMMARY

In normal dogs to which creatinine has been administered the creatinine clearance exceeds the xylose (glomerular) clearance by 15 to 40 per cent. This excessive excretion of creatinine is interpreted as due to the tubular secretion of this substance from the post-glomerular blood.

The tubular secretion of creatinine appears to be a linear function of the plasma concentration below 50 mgm. per cent, and therefore the tubular clearance is independent of the plasma concentration. Since the glomerular clearance is theoretically independent of the plasma concentration, it follows that the total creatinine clearance is likewise independent of the plasma concentration.

Changes in the glomerular clearance effected by diet and by erythroltetranitrate are accompanied by nearly proportional changes in the same direction in the creatinine clearance, indicating that the former are accompanied by nearly proportional changes in blood flow to the tubules.

Phlorizin completely arrests the tubular secretion of exogenous creatinine so that the total creatinine clearance is reduced to the level of the xylose (or glucose) clearance.

The excretion of endogenous creatinine is discussed briefly in the text.

Protocols accompanying tables 1, 2, and 3. (With the experiments on dog 36 arranged in sequence)

Experiment 140. Dog 36. April 8th. Weight 16.2 kgm., S. A. 0.72 sq. m. Cracker meal diet since March 16th. Twenty-four grams xylose in 640 cc. water by stomach at 9:10 a.m. Eight grams xylose in 320 cc. water by stomach and 8 grams creatinine in 100 cc. water subcutaneously at 9:45 a.m. Period 1 began at 10:42 a.m. Blood drawn at middle of each urine collection period.

Experiment 141. Dog 36. April 11th. Procedure same as in experiment 140 except that 6.4 grams creatinine were given.

Experiment 143. Dog 36. April 15th. Procedure same as in experiment 140 except that 4.0 grams creatinine were given.

Experiment 147. Dog 36. April 25th. Fed 4 lbs. meat April 18. Ate very little meat thereafter. Ate mixed diet April 22nd. Would not eat on April 23rd and 24th because of hot weather. On April 25th ate 850 grams raw beef at 7:20 a.m. Twenty-four grams xylose in 640 cc. water by stomach at 9:30 a.m. Eight grams xylose in 160 cc. water by stomach and 8 grams creatinine in 100 cc. water subcutaneously at 10:00 a.m. Period 1 began at 11:11 a.m. Blood drawn at middle of urine periods.

Experiment 149. Continuation of experiment 147. Thirty-two grams xylose in 200 cc. water by stomach at 6:35 p.m. and 8 grams creatinine in 100 cc. water subcutaneously at 7:10 p.m. At 7:20 ate about 250 grams cooked liver. Period 1 began at 8:20 p.m. Bloods as before.

Experiment 158. Dog 36. May 30th. Mixed diet since April 25th. Meat offered May 26th-29th but did not eat well because of hot weather. Twenty-four grams xylose in 320 cc. water by stomach at 8:45 a.m. Sixteen grams xylose in 640 cc. water by stomach and 8 grams creatinine in 100 cc. water subcutaneously at 10:10 a.m. Period 1 began 11:12 a.m. Note A: at end of period 3 one grain of erythroltetranitrate subcutaneously and two grains by stomach with 150 cc. water. Bloods 5 and 6 interpolated. Hematocrit 31.5 per cent. Dog obviously affected by erythroltetranitrate; weak at end of experiment.

Experiment 159. Dog 36. June 3rd. Cracker meal diet since May 30th. Five hundred cubic centimeters water had been given by stomach every day. Twenty-four grams xylose in 640 cc. water by stomach at 8:00 a.m. Eight grams xylose in 320 cc. water by stomach and 4.8 grams creatinine in 75 cc. water subcutaneously at 9:30 a.m. Period 1 began at 9:30 a.m. Note A: 16 grams xylose in 640 cc. water by stomach at 12:30 a.m. Twelve grams xylose in 320 cc. water by stomach at 1:00 p.m. Bloods drawn near middle of each urine period and interpolated to exact middle. True plasma creatinine determined as described in appendix. Non-creatinine chromogenic substance in plasma averaged 0.5 mgm. per cent, expressed as creatinine.

Experiment 160. Dog 36. June 7th. Cracker meal diet since May 30th. Twenty-four grams xylose in 640 cc. water by stomach at 9:00 a.m. Eight grams xylose in 320 cc. water by stomach and 8 grams creatinine in 100 cc. water subcutane-

ously at 9:30 a.m. Period 1 began at 10:34. Note A: at end of period 3, 3.2 grams phlorizin in $NaHCO_3$ solution intravenously and same quantity subcutaneously. Bloods drawn near middle of each urine period and interpolated to exact middle.

Experiment 157. Dog 43. May 27th. Weight 20 kgm., S. A. 0.92 sq. m. Cracker meal diet since May 6th. Thirty grams xylose in 800 cc. water by stomach at 8:30 a.m. Twenty grams xylose in 400 cc. water by stomach and 10 grams creatinine in 120 cc. water subcutaneously at 9:00 a.m. Period 1 began at 10:21 a.m. Note A: at end of period 3 one grain erythroltetranitrate subcutaneously and one and one-half grains, together with 10 grams xylose and 5 grams creatinine in 200 cc. water by stomach. Bloods drawn at middle of each urine period.

Experiment 155. Dog 30. May 23rd. Weight 20 kgm., S. A. 0.86 sq. m. Cracker meal diet since May 6th. Thirty grams xylose in 800 cc. water at 8:15 a.m. Thirty grams xylose in 400 cc. water by stomach and 20 grams creatinine in 200 cc. water subcutaneously at 9:00 a.m. Period 1 started at 10:00 a.m. Note A: at end of period 3, 4 grams phlorizin in $NaHCO_3$ solution intravenously and same quantity subcutaneously. Note B: at end of period 6, 4 grams phlorizin in $NaHCO_3$ solution intravenously. Bloods drawn at middle of urine periods. Dog vomited in period 4 and period 7.

APPENDIX. The method which we have used for the determination of true creatinine is that of Folin (1919) with a simple modification of the procedure used by Gaebler (1930) in which creatinine is absorbed in Lloyd's reagent. Our method is described in full below:

Total chromogenic substance. Ten cubic centimeters of a 1:10 tungstate filtrate of plasma (prepared by adding 7 volumes of water, one volume of 5 per cent sodium tungstate and one volume of 0.33 N H_2SO_4 to one volume of plasma) or a quantity of diluted urine containing from 0.2 to 0.8 mgm. of creatinine, are placed in 20×200 mm. test tubes and made up to a total volume of 10 cc. Standard creatinine solutions are prepared in the same manner. Five cubic centimeters of alkaline picrate, freshly prepared by adding 1 volume of 10 per cent NaOH to 5 volumes of freshly saturated, filtered picric acid solution are added to each tube and thoroughly mixed. Colorimetric comparison is made after 12 minutes. The picric acid used is recrystallized by Benedict's (1929) method, and the saturated solution prepared by mechanical shaking just before use.

Non-absorbable chromogenic substance. Ten cubic centimeters of a 1:5 tungstate filtrate of plasma, or a quantity of urine containing not over 5 mgm. of creatinine are placed in a 15 cc. centrifuge tube with sufficient water to bring the total volume to 12 cc. One drop of concentrated HCl and about 300 mgm. of Lloyd's reagent (measured roughly from a small spoon) are added to each tube; the tubes are stoppered with close fitting stoppers and shaken for 10 minutes. (Standard creatinine solutions are treated in the same manner for subsequent use, as described below.) The Lloyd's reagent absorbs the creatinine and leaves only the non-creatinine chromogenic substance in the solution. The tubes are centrifuged until the sediment is well packed, the supernatant fluid is then decanted into convenient receptacles and the empty tubes are left inverted to drain for subsequent use. For the determination of the non-absorbable chromogenic substance 10 cc. of the decanted fluid are placed in a 25 or 50 cc. conical centrifuge tube. Standards containing 0.001, 0.002, 0.003, etc., mgm. of creatinine are prepared in 10 cc. of water and placed in similar centrifuge tubes to facilitate preliminary comparison. Five cubic centimeters of alkaline picrate are added, and during the development of color the unknowns are centrifuged to throw down the slight precipitate which is produced by the alkali. Colorimetric comparison is made as usual after 12 minutes. A transient greenish color develops in the plasma filtrates so treated, but this disappears after 3 to 5 minutes and does

not interfere with the subsequent matching. The quantity of apparent chromogenic substances as determined colorimetrically must be multiplied by 12/10 to allow for the 10 cc. aliquot removed from the 12 cc. of extracted fluid. We find that 300 mgm. of Lloyd's reagent will completely remove 15 mgm. of creatinine. (After extraction of creatinine (our sample) there remains a slight residuum of chromogenic substance amounting to less than 0.5 per cent of the original creatinine; since our sample of Lloyd's reagent does not give any perceptible chromogenic blank by the above method, we interpret this residuum to be a non-absorbable chromogenic impurity in the creatinine.)

This method has been tested on a sample of urine which was found by a preliminary analysis to have 0.0023 mgm. per cubic centimeter of non-absorbable chromogenic substance; the recovery from 2, 4, 6 and 10 cc. of this urine was 0.0047, 0.0083, 0.0141 and 0.0240 mgm., showing a good proportionality between the color development and the quality of urine taken for analysis.

Recovery of creatinine from the Lloyd's reagent. The creatinine absorbed on the Lloyd's reagent in the above analysis is recovered by the addition of 12 cc. of water and 100 mgm. of MgO to the sediment in each tube. The sediment is loosened with a stirring rod, and the tubes stoppered and shaken for 10 minutes. There is no need to wash the sediment before liberating the creatinine because the included water is small in amount and contains only the now relatively dilute non-absorbable chromogenic substance. The suspensions are then centrifuged, decanted, and creatinine determined as above on 10 cc. aliquots. Known quantities of creatinine, as suggested above, should be included in the series and treated in an identical manner in order to evaluate the dilution resulting from the water included in the sediment. We recover about 75 per cent of the absorbed creatinine, as contrasted to the theoretical 83.3 per cent expected from the 10/12 aliquot used. The lower recovery is due, of course, to dilution by the water included in the Lloyd's after centrifuging.

Gaebler (1930) recovered from Lloyd's reagent which had been shaken as above with tungstate and picrate filtrates of dog's plasma more creatinine than had been absorbed, as calculated from the difference in the filtrates before and after extraction, and he concluded that the blood contains a non-chromogenic substance (other than creatine) which is converted to creatinine by the treatment with the Lloyd's reagent. We are not primarily interested in this substance here because, so far as glomerular filtration is concerned, only the preformed creatinine in the plasma is significant. The preformed creatinine is given, so far as it can be determined at the present time, by the difference in total chromogenic substance before and after extraction with Lloyd's reagent. The method which we describe above cannot be considered to be more than tentative because, as Gaebler points out, it is not known whether the color produced in untreated filtrates containing both true creatinine and other chromogenic substances is additive. But our immediate problem required a more exact analysis of the creatinine-like substances in plasma than is possible with the Jaffe reaction alone, and we believe that as a first approximation this method serves our purpose.

Interference of xylose in creatinine determination. When 3 mgm. of xylose in 5 cc. of water are mixed with 5 cc. of alkaline picrate no color develops within 30 minutes; 6 mgm. of xylose give a color perceptibly darker than a blank within this time, and 15 mgm. of xylose give a marked color within 15 minutes.

When 0.05 mgm. of creatinine and 2.5 mgm. of xylose in 5 cc. of water are mixed with 5 cc. of alkaline picrate about 4.0 per cent enhancement of color is observed over a control without xylose at the end of 30 minutes. These proportions (1 mgm. of creatinine to 50 mgm. of xylose) therefore represent the maximal which can be used

with safety if readings are to be made from 12 to 20 minutes after the picrate is added. The highest proportion in any of our experiments is 1:34 (plasma, period 8, expt. 159) and the proportion is usually much less than this.

Because the creatinine:xylose ratio exceeds the above, it is impossible to determine either the creatinine or the non-creatinine chromogenic substance normally present in the plasma and urine when these contain large quantities of xylose, and it is for this reason that we have made observations on endogenous creatinine excretion only in dogs which had not received xylose.

Sugar method. In extension of the sugar methods described by Jolliffe, Shannon and Smith (1932) emphasis may be placed upon a few points: the individual tubes of yeast used for glucose absorption must be centrifuged in an identical manner, and we find that increased accuracy in the U/P ratio can be obtained by handling the corresponding plasma and urine tubes together in each step; i.e., in the preliminary packing, in the absorption, in the final centrifuging and in the water bath. We believe that the Folin sugar method is unsafe when more than 0.4 mgm. or less than 0.15 mgm. of glucose (or the glucose equivalent of xylose) is used. The pipettes used for measuring standards and unknowns should be cleaned in cleaning fluid after each use to insure uniform drainage.

We find that creatinine, added to plasma which is subsequently precipitated with Somogyi's copper method and cleared of glucose by yeast, shows a reduction of 10 per cent in the Folin sugar method (i.e., 10 mgm. creatinine = 1.0 mgm. glucose). The error in the xylose determination due to the reducing power of creatinine is compensated in the U/P ratio, however, when the urine is so diluted that the quantities of xylose in plasma and urine are about the same. Since we try to dilute the urine so that it can be read against the same and identical standard as the plasma, the error is reduced in nearly all of our figures to 1.0 per cent or less and no correction has been applied for it. The xylose values in our tables are given as read against a glucose standard, no correction being made for the diffusion of xylose into the yeast or for its specific reducing power. The diffusion of xylose into the yeast is considered in calculating the glucose content, however.

Note: Our attention has been called by the authors to a paper by McCance and Madders (1930) on the absorption of sugars from the human intestine, which we had unfortunately overlooked. McCance and Madders suggested that the excretion of non-metabolized sugars might be used to measure the rate of glomerular filtration, and by assuming that the sugar which is retained in the body after injection is uniformly distributed throughout the body-fluids (presumed to be 60 per cent by weight) in order to ascertain the concentration in these fluids, they calculated from the rate of sugar excretion that the glomerular filtrate must amount to 100 to 178 cc. per minute. Plasma concentrations were not determined, and no comment was made on the possible secretion or reabsorption of the sugar, but it was noted that the figures so obtained were in good agreement with those obtained by Rehberg (1926) from the rate of excretion of creatinine.

We regret that we were unaware of McCance and Madders' paper when we prepared our own on the excretion of non-metabolized sugars.

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SOME MECHANISMS INVOLVED IN THE REGULATION OF THE CIRCULATION

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By means of the injection method (1, 2, 3, 4, 5, 6) one is able, in a single experiment, to determine the cardiac output (F), the stroke volume (SV), the mean (MCT), total and lesser circulation times, the volume of actively circulating blood in the heart, lungs and great vessels (V), and the total blood volume of man or of the dog.

The purpose of this investigation is to make determinations of these quantities in dogs, under the influence of cardio-vascular drugs, and under the influence of the arteriovenous fistula. There is no desire to analyze the response of the animal to therapeutic doses of the drugs involved, but rather to bring forward such considerations as may increase our understanding of the mechanism by which the circulation is controlled.

The experiments were performed as described in the above references. One hundred milligrams of "Brilliant Vital Red" were injected into the jugular vein. Consecutive samples were taken from the exposed femoral artery by means of a needle so bent that the sharp end was parallel to the artery and the other end pointed downward into the sampling tubes on the edge of a kymograph drum.

It was found that in cases where the serum was contaminated with hemoglobin, other pigments or with lipoids, that the colorimetric procedures could be carried out best when the small sample of plasma was diluted in 1 cc. of alcohol. When the alcoholic samples were stoppered and centrifugalized the precipitated proteins carried down all adventitious color and the dye in the alcoholic solution was proportional to the concentration of the dye in the plasma or serum. The lipoids made a clear solution in the alcohol and hence did not hinder colorimetry.

The results of these experiments are expressed in terms of surface area of the animal, as calculated from the formulae of Cowgill and Drabkin (7). Due to the fact that it was impossible for us to work with trained, unanesthetized dogs, it was thought that a dose of morphine, about one milli-

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gram per kilogram, would give conditions sufficiently near the basal (8, 9) to use as a starting point. The dogs were in a post-nutritive state.

Figure 1 is a typical time concentration curve of consecutive samples taken from the femoral artery of a dog under morphine alone, when 100

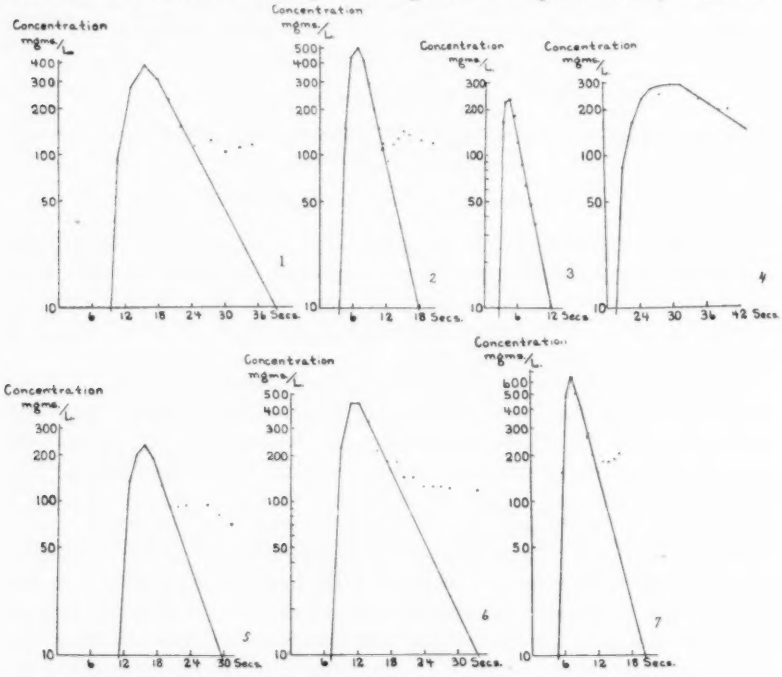

Fig. 1. Time concentration curve of dye in arterial blood after injecting 100 mgm. of Brilliant Vital Red into jugular vein. Dog under morphine; table 1, dog 2.

Fig. 2. Same as figure 1. Morphine; fast circulation; table 2, dog 6.

Fig. 3. Same as figure 1. Dog under morphine and atropine; table 3, dog 3.

Fig. 4. Same as figure 1. Morphine; 0.5 mgm. adrenin given intravenously 20 seconds before dye was injected. Table 4, dog 6.

Fig. 5. Same as figure 4. Adrenin given 60 seconds before dye. Table 5, dog 6.

Fig. 6. Same as figure 5. Morphine and atropine. Adrenin 20 seconds before dye. Table 6, dog 8.

Fig. 7. Same as figure 1. Inhalation of amyl nitrite begun 20 seconds before dye was injected. Table 8 dog 6.

mgm. of Brilliant Vital Red had been injected quickly into the jugular vein. Table 1 indicates the results of several similar experiments on other dogs. The slow heart involves a large stroke volume. This, in turn, means that the diastolic size of the heart is large and that the filling

TABLES 1-9

Circulatory constants under experimental conditions

TABLE NUMBER	EXPERIMENTAL CONDITIONS	DOG NUMBER	SURFACE AREA	LESSER CIRCULATION TIME	MEAN CIRCULATION TIME	TOTAL CIRCULATION TIME	PULSE PER MINUTE	FLOW PER SQ. M.	"V" PER SQ. M.	SV PER SQ. M.	V/SV
			sq.m.	*sec.*	*sec.*	*sec.*		*liters*	*liters*	*cc.*	
I	Morphine with slow heart	1	0.48	8.5	19.7	19.0	33	3.79	1.355	114.5	11.73
		2	0.43	8.7	19.0	15.5	39	3.47	1.090	88.8	12.70
		3	0.71	4.2	13.5	14.0	51	4.50	1.010	88.0	11.50
		4	0.60	7.2	13.8	12.0	54	4.40	1.085	82.0	13.20
		av.		7.15	16.5	15.1	44.3	4.04	1.135	93.3	12.20
II	Morphine with fast heart	7	0.68	5.9	11.5	11.9	96	6.2	1.19	64.6	18.5
		6	0.44	3.4	8.1	9.8	105	5.5	0.74	52.5	14.1
		av.		4.7	9.8	10.9	101	5.9	0.97	58.6	16.5
III	Morphine and atropine	1	0.48	3.3	7.6	9.7	165	8.26	1.450	44.4	32.7
		2	0.43	3.8	8.7	10.0	93	4.42	0.632	40.7	15.5
		3	0.71	3.2	6.0		120	10.50	1.050	87.5	12.0
		8	0.58	3.0	5.8	6.0	120	10.00	0.950	83.1	11.4
		av.		3.3	7.0	8.6	125	8.30	1.021	63.9	16.0
IV	Morphine, adrenin 20 seconds before dye	1	0.48	22.0	41.0	20.0	51	1.58	1.08	31.1	34.8
		2	0.43	14.0	26.6	17.0	60	2.37	1.05	39.5	26.6
		3	0.71	19.5	41.6		75	2.92	2.02	38.9	52.4
		6	0.44	19.5	41.0	21.5	57	1.84	1.38	31.8	43.4
		av.		18.8	37.6	19.5	61	2.18	1.38	35.3	39.1
V	Morphine, adrenin 60 seconds before dye	2	0.41	12.3	35.1	18.2	60	2.04	1.190	33.9	35.1
		5	0.42	14.9	43.3	29.1	54	1.95	1.410	36.2	39.0
		av.		13.6	39.2	23.7	57	2.00	1.300	35.1	37.0
		1	0.48	7.0	14.3	12.0	63	9.85	2.350	156.0	15.1
		6	0.42	6.9	14.5	13.1	69	3.84	0.924	55.5	16.7
		7	0.68	11.3	18.2	10.0	66	5.06	1.590	76.0	20.9
		av.		8.4	15.7	11.7	66	6.25	1.620	95.8	16.9
VI	Morphine-atropine, adrenin 20 seconds before dye	5	0.45	7.0	16.3	9.3	165	3.65	0.99	22.0	45.0
		8	0.51	7.2	15.0	10.8	105	3.07	0.77	29.2	26.4
		9	0.70	9.0	23.5	20.0	156	2.30	0.90	14.7	61.2
		av.		7.7	18.3	13.4	142	3.01	0.89	22.0	40.4
VII	Morphine-atropine, adrenin 90 seconds before dye	5	0.43	5.7	9.1	5.9	117	4.92	0.753	42.5	17.7
		3	0.71	8.5	13.9	7.8	93	6.00	1.390	64.3	21.6
		av.		7.1	11.5	6.9	105	5.46	1.072	53.4	20.0

TABLES 1-9—*Concluded*

TABLE NUMBER	EXPERIMENTAL CONDITIONS	DOG NUMBER	SURFACE AREA	LESSER CIRCULATION TIME	MEAN CIRCULATION TIME	TOTAL CIRCULATION TIME	PULSE PER MINUTE	FLOW PER SQ.M.	"V" PER SQ.M.	SV PER SQ.M.	V/SV
			sq.m.					*liters*	*liters*	*cc.*	
VIII	Morphine, amyl nitrite 20 seconds before dye	5	0.44	3.7	9.2	9.3	111	5.80	0.889	52.0	17.1
		6	0.44	4.8	9.7	8.3	108	4.59	0.745	42.5	17.5
		8	0.54	6.2	11.5	10.0	90	5.93	1.130	66.0	17.1
		av.	0.47	4.9	10.1	9.2	103	5.44	0.921	53.5	17.2
IX	Dog with A.V. fistula										
	Before fistula	4	0.60	7.2	13.8	12.0	54	4.40	1.085	82.0	13.20
	After A.V. fistula, morphine alone	4	0.58	4.0	10.9	12.0	42	5.60	1.020	133.0	7.67
	Fistula morphine atropine	4	0.60	4.0	7.8	10.0	140	5.95	0.774	42.5	18.20
	Fistula morphine-adrenin 20 seconds before dye	4	0.63	20.5	30.1	15.5	51	2.70	1.350	53.0	25.40
	Fistula morphine-adrenin 60 seconds before dye	4	0.60	14.7	19.6	8.2	90	7.30	2.380	81.2	29.30

pressure on both sides of the heart is increased (6). Since the increased filling pressure of the left heart is communicated through the pulmonary capillaries, they should be stretched and have a relatively large capacity. This is corroborated by the fact that the calculated volume of blood in the heart, lungs and great vessels (V) is relatively large. This, in turn, is derived from, and consistent with, the relatively long mean circulation time.

In table 2 and in figure 2, we have illustrated experiments in which the morphine did not take effect as well as usual. The dogs were restless and the heart more rapid. The excitement induced increased cardiac output, but the rapid heart resulted in a smaller stroke volume. The volume of blood in the heart, lungs and great vessels may be reduced because the rapid heart pumps blood out of the lungs and a lesser filling pressure is necessary with the smaller stroke volume. These factors combined result in decreased circulation times.

In figure 3 and table 3 are illustrated experiments in which the usual dose of morphine was combined with one or two milligrams of atropine—enough to accelerate the heart and eliminate respiratory arrhythmia.

We had expected from previous work (8, 9) that atropine would not

increase markedly the cardiac output. In one case the flow was low (dog 2) with a reduced stroke volume and diminished blood in the heart and lungs (V). In the other three cases there was a large stroke volume in spite of the rapid heart rate. The very short circulation times indicate a reduced or normal central blood volume (V). The variability of cardiac output under these conditions will have to be explained as a result of further work in which other factors are taken into account.

Various scattered observations have been made upon the effects of adrenin and similar drugs (11, 12, 13, 14, 15, 17, 18) upon the heart output and the circulation time. Here the attempt is made to correlate the effect of adrenin upon the several aspects of hemodynamics in the single animal.

When 0.5 mgm. adrenin is given intravenously 20 seconds before the experiment is made we have concentration curves which are strikingly like those in cases of decompensated heart disease. A typical curve is given in figure 4 and the results of the experiments are summarized in table 4. There is intense vasoconstriction, high arterial pressure, and a slow heart due to the depressor reflexes emanating from the aortic arch and the carotid sinus. The peripheral constriction serves to markedly reduce the cardiac output. This occurs in spite of the fact that the blood pressure is high and the heart is working under considerable strain. Although the heart is slow the reduced output gives rise to a low stroke volume. That the diastolic size of the heart, under these conditions, is increased can be deduced from the fact that the volume of actively circulating blood in the heart, lungs and great vessels is relatively large. This large V indicates increased intrapulmonary pressure and, in combination with the low flow, gives rise to the tentative hypothesis that there may be blood completely stagnating in the pulmonary tree unmixed with dye and hence not included in V. This should be added to V if we are to regard this quantity as an emergency reserve (6).

These experiments are of particular interest in relation to the results of similar experiments performed on cases of cardiac decompensation. In such cases, as well as in those under consideration here, the stroke volume is small, the diastolic size and quantity V large, and the circulation times markedly increased. In both types of case, the time concentration curve of the arterial dye is low and delayed. The ratio, that is, the number of beats necessary to clear the lungs and great vessels of blood, is in each case markedly increased. In both cases the inference is working under conditions which impose labors that are beyond the physiological limits of compensation. In heart disease the condition is relatively permanent and is due to myocardial weakness. In the other case, the condition is temporary and is due to the sudden increase in blood pressure and its reflex effects.

If we are justified in saying that during the activity of the depressor mechanism, the animal has mobilized its cardiovascular reserves for the

emergencies consequent upon the situation which causes these changes, we can postulate that the damming back of blood in the lungs is an important phase of the process. The next step in the carrying out of this process is indicated by the following experiments:

In table 5 and figure 5 experiments are illustrated in which the determinations were made 60 seconds after the injection of 0.5 mgm. of adrenin. In some of these experiments (dogs 2 and 5) the condition is exactly as it was in the previous experiments. The heart is slow, circulation times long and the curve flat and delayed, i.e., the cardiac mechanisms are unable to compensate. In other cases the depressor mechanism has let go, vasodilatation has evidently reduced the blood pressure and increased the flow, as has been shown to be the case when small doses of adrenin are given (12, 17, 18). The changes in V are correlated with the increased flow and increased stroke volume. This latter factor results in increased left ventricular filling pressure and pulmonary dilatation, and further, the increased flow might be expected to pick up and carry on, mixing with the dye, such stagnant blood as would exist in the lungs during the activity of the depressor mechanism. These factors together tend to reduce the circulation times and have a parallel in a similar set of conditions which exists during recovery from cardiac decompensation where the increased flow and reduced circulation times occur in spite of an increase in volume of the actively circulating blood in the heart, lungs and great vessels.

In order to find out whether the peripheral vasoconstriction or the slow heart rate immediately following adrenin, is responsible for the reduced cardiac output and other changes in the circulation, it was thought well to repeat the experiments with adrenin, on dogs which were morphinized and atropinized as well.

As is seen in table 6 and figure 6, the output is well below normal in spite of the rapid heart. The volume of actively circulating blood in the heart, lungs and great vessels, on the other hand, is reduced, giving circulation times well below the morphine-adrenin experiments. These factors are correlated with the markedly reduced stroke volume and serve to indicate whereas the volume flow of blood through the circulation is regulated peripherally, the degree of congestion in the heart, lungs and great vessels and hence in part the circulation times depend upon the activity (rate) of the heart.

There is an apparent contradiction here with the situation in the decompensated cardiac cases where, in spite of the rapid heart, the amount of blood in the heart, lungs and great vessels is markedly increased. This is to be explained by the fact that the diastolic size and filling pressure is in the one case (cardiac) high and in the other case low. In other words, the atropinized dog's heart, even under the influence of adrenin, is competent to pump blood into the circulation, whereas the heart of the de-

compensated cardiac can do so only under the stimulus of enough stretching to involve a high filling pressure and marked pulmonary congestion. Atropine helps to compensate the normal heart to the task set by adrenin but could not be expected to help compensate the weakened heart to the task set by heart disease.

If the experiment is performed 90 seconds after the adrenin is given, the atropinized dog responds with an increased flow, "V," and stroke volume. The peripheral constriction has abated and the heart is being regulated accordingly (see table 7).

In order to observe the effect of an opposite set of conditions, the dogs were given amyl nitrite, one ampoule, by inhalation. The experiment was performed 20 seconds later. The results are seen in table 8 and figure 7. This drug increases the heart rate and causes a marked reduction in the blood pressure through its effect of relaxing the arterioles. The flow per minute is increased perhaps significantly above the normal. The markedly reduced circulation times give rise to the conclusion that, under these conditions, the heart is able to reduce the central congestion (V) well below ordinary limits. In other words, the heart pumps the blood out of the lesser circulation and into the greater.

A similar set of conditions is involved when an arteriovenous fistula is made connecting large vessels. Prof. C. E. Bird has kindly made a fistula between the femoral artery and vein of dog 4, and allowed us to experiment with the animal. The results of these experiments are given in table 9. In the first experiment under morphine, the heart is slow and the output increased (see dog 4, table 1). The quicker circulation times leave the quantity V very similar to that found in the normal dog under morphine. When the dog with the fistula is given morphine and atropine, the heart rate is greatly increased but there is no marked increase in output. Circulation times are reduced and there is a decrease in the quantity V. The situation as a whole is almost, if not quite parallel to the situation in the normal dog after amyl nitrite.

When the dog with the fistula is given the usual dose of adrenin, the change is very similar to that produced by adrenin in the normal dog. The greatest differences are traceable directly to the fistula and involve increases in flow and in the quantity V.

It is a pleasure to recognize the technical assistance of Mr. J. H. Rompf.

SUMMARY

1. An analysis is attempted, by means of the injection method, of the relations between the cardiac output, the circulation time and the volume of actively circulating blood in the heart, lungs and great vessels, in response to cardiovascular drugs and the arteriovenous fistula.

2. Morphine slows the velocity of the circulation, increases the central blood volume and probably leaves unchanged the volume flow.

3. Atropine increases the velocity of the circulation and either increases the volume flow or leaves it unchanged. In the latter case there is a marked reduction in the central volume of active blood.

4. Adrenin at first (depressor effect) reduces both the velocity and the volume flow to such a degree that the findings are similar to those in decompensated heart disease. As the effect of the drug wears off, the volume flow and velocity are both markedly increased.

5. The fact that after atropine, adrenin causes a reduced flow in spite of the rapid heart, but gives a small central blood volume and hastens the velocity of the circulation, indicates that the volume flow is to a great extent a function of peripheral constriction and that the central active blood volume and hence, in part, the circulation times are a function of the heart's activity (rate).

6. Amyl nitrite increases the flow and the velocity of the circulation. Under this drug and with the A.V. fistula as well, the heart pumps blood from the lesser into the greater circulations, giving a markedly reduced central active blood volume.

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MOVEMENTS OF THE BASE OF THE VENTRICLE AND THE RELATIVE CONSTANCY OF THE CARDIAC VOLUME

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The view that the heart remains relatively constant in volume in diastole as in systole, has been maintained by one of us on the basis of an analysis of the cardiopneumogram (1). Thus cardiac volume changes produce pressure changes within the lungs which, when transmitted through the respiratory tree and corrected for the elastic give of the chest walls, seem to be produced by a cardiac volume change of 1 or 2 cc., (certainly less than 7.5 cc.) and last through only a part of systole. This has been confirmed in part by E. Holzlöhner (2) who admits that the net systolic decrease in intrathoracic blood volume is small (5 cc.) but insists that much larger quantities of pulmonary air (25–30 cc.) are displaced by changes in intrathoracic blood volume during the course of systole. That the quantities of air which move in and out of the chest during the cardiac cycle are of the order of 1 or 2 cc., is easily demonstrated by a simple experiment. If one fills the mouth with smoke and maintains respiratory standstill with the internal nares closed and the glottis open, a tiny puff of smoke is seen to issue from the lips toward the end of each ventricular systole. The amount of this is usually not more than 0.5 cc. as contrasted with Holzlöhner's figure of 25 to 30 cc.

Teleologically one might well expect that the heart is organized so as to move blood and blood alone. It does not waste energy moving the contiguous tissues about. The heart is able to pump blood without much displacement of contiguous tissues through the fact that its major pumping action is due to the caudo-cephalad movement of the atrioventricular septum. This in turn insures the reciprocal action of the two chambers so that the auricles fill during ventricular systole, and ventricular filling occurs at the expense of a reduction in the volume of the auricle.

It has long been known that the base of the ventricle makes a large excursion toward the apex during systole and that the apex makes little move-

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This paper is from a thesis submitted by Mr. Rompf in partial fulfilment of the requirements for the Master's degree at the University of Louisville.

ment at any time (Da Vinci, 3). Though the truth of these observations has not been questioned, their significance in regard to the time of auricular filling has not been fully appreciated.

Movements of the base of the heart. In order to make further inquiry into the nature of the cardiac movements, records were taken of the caudo-cephalad movements of the base and apex of the ventricle in the frog, turtle and dog.

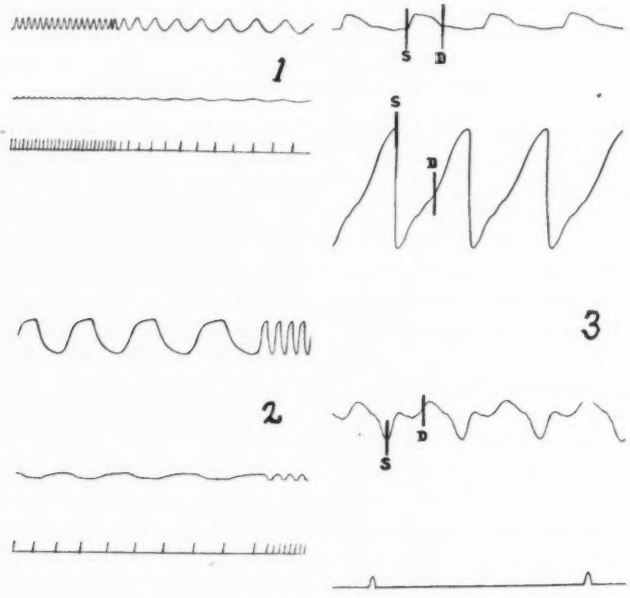

Fig. 1. Mechanically recorded graphs of the movement of the ventricular base of the frog's heart, upper curve, and of the ventricular apex, middle curve. Lower curve, time in seconds. Downstroke indicates a caudad movement. Magnification of levers is 2:1.

Fig. 2. The same as figure 1—turtle heart.

Fig. 3. Dog heart similar to figure 1. Upper curve, pulse; second curve, movements of ventricular base; third curve, movements of ventricular apex; fourth curve, time in seconds.

The movements were recorded by means of light, delicately counterpoised L-shaped balsa-wood levers. On the end of the upright arm a small barbed hook was pinioned so that it could be thrust into the A.V. groove or into the apex of the heart in such a way as to record only cephalo-caudad movements. The records were taken on cellophane, smoked with a kerosene lamp and fixed in thin shellac (cf. 4). They could be printed photographically with great ease and clearness.

As seen in figures 1, 2 and 3, in all three animals the base of the heart moves downward toward the apex during systole and the apex makes very slight movements. The frenulum which is more or less directly anchored to the anterior abdominal wall, plays an important part in fixing the apex of the turtle heart, for when it is cut the movements of the apex are greatly increased and those of the base greatly decreased. The frenulum of the bullfrog plays a much less important rôle since cutting this strand of connective tissue which is sometimes attached to the posterior surface rather than to the apex, causes little change in the normal movements of the heart.

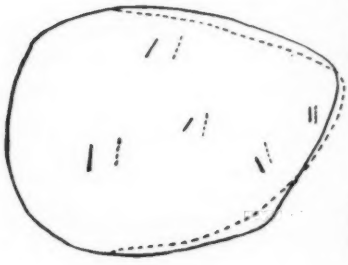

Fig. 4

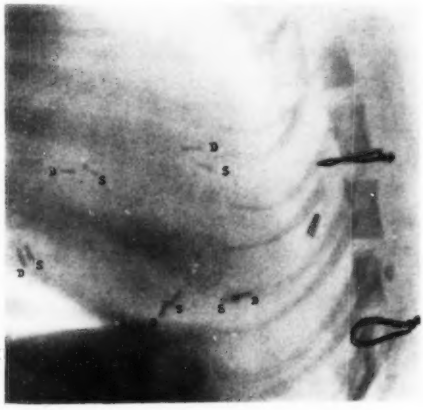

Fig. 5

Fig. 4. Fluoroscopic tracing of diastolic (solid line) and systolic (dotted line) portions of the cardiac outline and of metallic objects placed in the ventricular myocardium. Healthy intact dog.

Fig. 5. X-ray picture of the same heart as figure 4, from a slightly different angle. The diastolic positions of the metallic objects are marked *D* and the systolic positions are marked *S*.

The dog has of course no frenulum. The forces which hold the apex stationary (in this dimension) have been analyzed (1). The most important of these perhaps is the recoil of the heart against the aortic pressure.

That these movements occur not only when the heart is exposed but also when it is in its normal relations in the thorax, is shown by visualizing the base and apex of the ventricle fluoroscopically. To do this the shaft of the barbed point of a fish-hook was inserted into a long needle. The needle was thrust through the chest wall of an etherized dog and under the fluoroscope the barb was dislodged in the myocardium at the base or apex of the ventricle. In other experiments the chest was opened aseptically and bits of silver wire were placed in the ventricular wall near the A.V. groove as well as near the apex.

When the animals had recovered from the immediate effects of these procedures they were perfectly normal, active and playful. With the dog on its side the fluoroscopic appearance was remarkable. To one who had been in the habit of thinking of the cardiac contractions in terms of the small undulations of the edges of the ventricular shadow, the movements of these foreign bodies seemed fantastically vigorous. Only their constancy from day to day, their regularity and the autopsy findings dispelled the notion that the metal objects were not washing about in the cardiac cavities.

As seen from the outline sketch (fig. 4) and the skiagram (fig. 5), the foreign objects approach the apex of the heart during each systole. The distance of approach is roughly proportional to the length of muscle between the object and the apex. The movements toward the apex are much greater than those toward the axis.

These findings emphasize the doubt cast upon the validity of cardiac output determinations derived from calculations which have as their basis the difference in area of the systolic and diastolic x-ray heart shadows (5, 6). The atria cannot be distinguished from the ventricles by x-ray. Therefore, the x-ray method gives us only an indication of the total heart volume change and not the volume change of the ventricles as would be needed for output determination. In order to determine the output of the heart by x-ray, it is necessary to visualize the movements of the top as well as of the sides of the ventricles. We might hazard the guess that calculations which take into account the shortening of the ventricle, the consequent thickening of its walls as well as their inward movement, would be rather complex. Bardeen's formula which is based upon measurement of the whole heart, is hardly applicable to such calculations.

Volume changes of auricles and ventricles. If the anterior body wall of a normal frog is carefully observed a slight pulsation can be seen. This slight movement is by no means comparable to the volume of blood which is forced from the heart at each beat. The tissues forming the body wall in front of the heart are so thin and flexible that one would expect them to follow changes in the cardiac volume. Since the movements impressed upon the anterior body wall by the heart are barely visible, one is led to the conclusion that the total heart volume remains nearly constant during the cardiac cycle.

When the heart is exposed a movement of the organs surrounding it would be observable at each systole if this were not the case. No such movements can be seen because the downward movement of the A.V. septum at each systole allows the auricle to fill during this period. The heart is thus enabled to expend its energy in moving blood instead of wasting some of it in moving the surrounding organs.

Evidence to substantiate this attitude was obtained from simultaneous

records of the volume changes of auricle and ventricle in the frog and from similar experiments on the turtle and dog heart.

The apparatus consisted of very delicate tambours which could record volume changes from glass cardiometers or from the pericardial cavity by

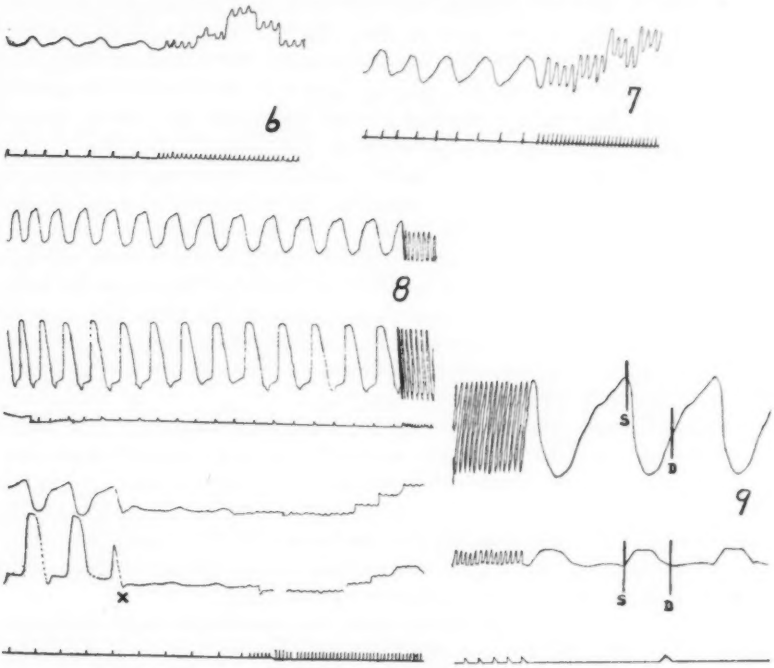

Fig. 6. Volume changes of the frog's heart as recorded from the pericardium. The "staircase" appearance is the calibration where 0.1 cc. portions of air were successively injected into the system and withdrawn. Time in seconds.

Fig. 7. Frog heart, cardiometer cup over the whole heart. Calibration as in figure 6. Time in seconds.

Fig. 8. Volume changes of the auricle, upper curve, and ventricle, middle curve, taken simultaneously. At *X* a tube connecting the two recording systems was opened. Calibration as in figures 6 and 7. The "steps" indicate about 0.05 cc. for each recorder. Time in seconds.

Fig. 9. Upper tracing, oscillations of pressure within the dog's pericardium (see text); middle tracing, pulse; lower tracing, time in seconds.

direct air transmission. A water manometer was connected into the system and pressure was maintained at a level lower than 2 mm. of water. The records could be quantitated by injecting, with a syringe, small quantities of air as they were being taken.

When the tambour was connected to record volume changes of the frog or turtle heart within the pericardial cavity, net changes were small—less than 0.1 cc. (see fig. 6). They were of the same order when a glass cardiometer was placed over the whole heart (fig. 7), but when separate cardiometers were placed over auricle and ventricle, the volume changes of each chamber were five to ten times as great (fig. 8). Furthermore, they were reciprocal, that is, when the auricles emptied the ventricles filled and *vice versa*. This is shown not only by careful examination of the records, but also by the results of opening a cross connecting tube which allowed air to flow from one recording system into the other.

We were unable to get simultaneous records of the auricle and ventricle in the turtle. However, by comparing the ventricular volume change (0.7 cc.) with that of the whole heart (0.1–0.05 cc.) it is easily seen that the auricle must be filling as the ventricle empties and *vice versa*. Due to the fact that the manipulations involved in these experiments on the turtle and frog probably interfere more with venous inflow than with arterial outflow, one is justified in assuming that in the intact animal the auricular filling would be more prompt and complete and would hence compensate even more closely the ventricular emptying.

A few attempts were made to repeat Stefani's (7) experiments in which he recorded the pressure changes within the partially inflated dog's pericardial cavity. The chest was open and the pressure and anatomical relationships which give rise to the normal auricular filling, were quite disarranged. Further, mechanical oscillations of the heart within the flexible pericardial cavity gave rise to oscillations of pressure which could not be rightly attributed to cardiac volume changes.

We did, however, obtain records which were closely like those of Stefani. Due to the fact that we took simultaneous pulse records we came to the conclusion that the rapid inflow phase (fig. 9) which Stefani attributes to "active diastole," really occurs, in part at least, during systole. If this is in fact a reflection of a true cardiac volume change, it seems reasonable to attribute it to auricular filling during systole. This is quite consistent with the fact that the auricular pressure is lowest in early systole (8), as well as with the fact that Burton-Opitz (9) observes a marked increase of jugular flow during ventricular systole.

SUMMARY

1. Direct recording of the movements of the base and apex of the heart of the frog, turtle and dog shows that at each systole the base of the heart makes a large movement toward the apex, while the apex remains almost stationary. This enables the heart to expend its total energy in moving blood instead of wasting part of it in moving the surrounding organs.

2. The same thing is shown in the normal intact dog when the base of

the ventricle is visualized fluoroscopically by means of small metal objects placed in the myocardium near the A.V. groove.

3. When volume changes of the frog's auricle and ventricle are recorded separately, they are seen to be reciprocal, the auricle fills as the ventricle empties and *vice versa*. Thus volume changes of the heart as a whole are very small.

4. Similar experiments on the turtle and dog indicate similar conclusions.

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STUDIES IN THE B VITAMINS

I. Statistical Comparison of Small and Large Litters of Rats on a Normal Stock Diet

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The work of this laboratory for the past several years has been concerned with the effects of nutritional deficiencies on reproduction, pregnancy, lactation, growth, and pathological conditions in the albino rat. For this reason it has been highly important to standardize carefully the stock litters from which our experimental animals are obtained. In previous years, in this, as in many other laboratories, six young per litter was considered a "normal" number. To assure comparative growth results and in no case to subject the nursing mother to undue strain, all litters of larger size were reduced to six young. In any controlled series of tests litter mates are preferable to less closely related individuals even in a highly inbred stock. It was therefore determined to study carefully the individuals of larger litters to see if experimental accuracy demanded the waste of animals entailed in the reduction of litters.

The problems presenting themselves were: 1, the obtaining of consistently large litters by careful selective breeding, and 2, the securing of data on *a*, comparative weight changes of mothers during lactation of large and small litters; *b*, comparative birth weights of young in large and small litters; *c*, comparative weaning weights of young in large and small litters; *d*, comparative weaning weights of young in large litters and those in large litters reduced in number early in the lactation period; *e*, comparative mortality in large and small litters.

This investigation covered a period of five years, and our statistics are compiled from records on 1,624 young normally born on general stock diet.

The stock of the laboratory was a mixture of a Portland albino rat strain with fifteen animals obtained from the Evan's laboratory at the University of California. In 1926, a few months before this investigation was begun, six rats were procured from the Johns Hopkins laboratory for interbreeding. At present we are able to trace the heredity of any given rat back to these original individuals.

Our animals are hardy, active, and tame. They are housed in large sawdust-bottomed cages that are well lighted, well ventilated, and clean. The diet consists of McCollum's grain mixture[1] using whole grain products, fresh minced vegetables, whole milk, and a small portion of table scraps. Cod liver oil[2] and iodine salts are supplied in addition to this diet.

To increase the size of litters our breeding animals were selected only from litters of nine or more young which showed approximately equal numbers of males and females. Only the most nearly perfect animals were chosen. The mothers were allowed to nurse their entire litters regardless of size for twenty-eight days. Within two years the mean number of young per litter was 9.26 as compared to 7.07 young per litter for the preceding year and a half, a significant increase. Following this period intensive selective breeding was discontinued, but the stock already obtained

TABLE 1

Size of litters, 1926–1931

YEARS	NUMBER OF LITTERS	NUMBER OF YOUNG	RANGE IN NUMBER OF YOUNG	MEAN NUMBER OF YOUNG	PROBABLE ERROR OF MEAN	STANDARD DEVIATION	RANGE / STANDARD DEVIATION	DIFFERENCE OF MEANS / PROBABLE $ERROR_{dif.}$
1926–1927	30	212	3–11	7.07	±0.27	±2.18	4.13	
1928–1929	72	667	2–14	9.26	±0.20	±2.50	5.20	6.64
1930–1931	87	745	2–16	8.65	±0.19	±2.60	5.77	4.79
1928–1931	159	1,412	2–16	8.99	±0.14	±2.59	5.79	5.27
1926–1931	189	1,624	2–16	8.59	±0.13	±2.61	5.62	5.07

Selective breeding of rats carried on in 1928–1929 produced the significant increase in the mean size of litters noted above. The decline in the mean for 1930–1931, when careful selection was discontinued, is interesting but cannot be considered significant. The final effect shown is a distinct change in the strain after 1927 when any of the year groups are compared.

was closely inbred. There was a subsequent decrease to 8.65 young per litter in the year and a half following. (See table 1.)

To determine whether the nursing of large litters placed undue strain on the mothers, careful weight records of the females during lactation were kept. Weight changes of the mother during each of the four weeks of lactation were considered indicative of her condition. These weight

[1] McCollum's grain mixture (ground whole grain products):

Wheat	1,000 parts
Corn	1,000 parts
Oats	1,000 parts
Flax seed	400 parts
Casein (unpurified)	400 parts
Calcium carbonate	20 parts

[2] Supplied by Mead, Johnson & Co., Evansville, Indiana.

changes were correlated with the number of young in the litters. Examination of table 2, in which the results are given, will show that the correlations are not high enough to be considered significant in any case. These

TABLE 2

Correlation of weight changes in mothers during lactation with number of young in litters

	NUMBER OF LITTERS	NUMBER OF YOUNG	RANGE	MEAN	PROBABLE ERROR OF MEAN	STANDARD DEVIATION	RANGE / STANDARD DEVIATION	CORRELATION	PROBABLE ERROR OF CORRELATION
One week:									
Number of young in litters			3–12	7.77	±0.18	±2.32	4.31		
	72	567						+0.15	±0.08
Weight changes (grams) in mothers			−40–+35	+0.07	±1.07	±13.50	5.56		
Two weeks:									
Number of young in litters			3–14	7.65	±0.20	±2.38	5.04		
	68	532						+0.23	±0.08
Weight changes in mothers			−50–+25	−4.52	±1.59	±19.38	3.87		
Three weeks:									
Number of young in litters			3–14	7.56	±0.20	±2.41	4.98		
	63	488						−0.07	±0.09
Weight changes in mothers			−50–+30	−5.63	±2.17	±20.33	3.94		
Four weeks:									
Number of young in litters			3–14	8.16	±0.23	±2.40	5.00		
	51	416						−0.06	±0.09
Weight changes in mothers			−65–+50	−4.39	±2.15	±22.76	5.05		

The correlations approach zero and in no case are high enough to be considered significant. These results indicate that large litters do not of themselves subject the nursing mother to undue strain.

results indicate that in our animals the nursing of large litters does not of itself subject the mother to undue strain.

To determine the effect on the young of the production of large or small litters, the weight records from birth to weaning were studied, as were the mortality records for the nursing period (fig. 1.)

TABLE 3

Correlation of weight of young at birth with number of young in litters

	NUMBER OF LITTERS	NUMBER OF YOUNG	RANGE	MEAN	PROBABLE ERROR OF MEAN	STANDARD DEVIATION	RANGE / STANDARD DEVIATION	CORRELATION	PROBABLE ERROR OF CORRELATION
Number of young in litters	164	1,440	2–16	8.78	±0.12	±2.28	6.58	−0.21	±0.05
Birth weight in grams			3.7–6.5	5.27	±0.03	±0.58	5.00		

The negative correlation of 0.21 is small but probably reliable. This indicates that an increase in the size of the litter may tend to be accompanied by a small decrease in birth weight.

TABLE 4

Correlation of weight gains to weaning with number of young in litters

	NUMBER OF LITTERS	NUMBER OF YOUNG	RANGE	MEAN	PROBABLE ERROR OF MEAN	STANDARD DEVIATION	RANGE / STANDARD DEVIATION	CORRELATION	PROBABLE ERROR OF CORRELATION
I. Using all litters available									
Number of young in litters	67	522	1–15	7.79	±0.26	±3.10	4.84	+0.15	0.08
Weight gains (grams), 28 days			15–59	31.70	±0.90	±10.92	4.12		
II. Using only large litters and large litters reduced in size early in the lactation period									
Number of young in litters	49	438	1–15	8.94	±0.26	±2.64	5.68	+0.21	0.09
Weight gains (grams), 28 days			15–51	32.45	±1.03	±10.71	3.46		

In I weight gains in litters of all sizes are considered. In II reduced and unreduced large litters are considered. Both parts of the table show small positive correlations, which, however, cannot be considered significant. These results therefore indicate that young from large litters show growth gains which are entirely comparable to those of young from smaller litters.

The results of the comparison of the average birth weights with the number of young in the litter are given in table 3. A small but probably reliable negative correlation was found, indicating that an increase in the

size of the litter tends to be accompanied by a small decrease in birth weight.

In table 4 are given our statistical data on weight gains to weaning, 28 days. A correlation was first run between the average weight gains and the number of young per litter at weaning on all animals for which records were available up to 1930. (At that time breeding to increase the weights of the individual animals was begun, so the weights for 1930–1931 are not comparable and are not included.) The results showed a small positive correlation, tending to favor the large litters, but too small to be considered significant. These findings indicate that growth in the large litters was definitely normal for our strain.

To see if the young of large litters were being permitted optimal growth, a second correlation was run. Litters of eight or more young were taken, and in about half of these the number of young was reduced early in the

TABLE 5

Mortality

LITTER SIZE	NUMBER OF LITTERS	NUMBER OF YOUNG	MORTALITY NUMBER	MORTALITY PER CENT
Small (1–4 young)	14	46	8	19.0
Medium (5–8 young)	68	459	22	4.5
Large (9–12 young)	101	1,033	43	4.2
Very large (13–16 young)	6	86	7	8.1

The death rate is lowest in the large and medium-sized litters and increases in both the small and the very large litters in our series.

lactation period. In this correlation (see table 4, II) the gains of reduced litters are no greater than the gains of the unreduced litters.

An examination of our data on mortality of young during the lactation period indicates a tendency toward a reversed curve—a greater death rate in the small and in the very large litters (fig. 1 and table 5).

In the small litters (1 to 4 animals) the number of young may not be sufficient to give an accurate picture of the death rate, and it is not improbable that if the data were increased the percentage of mortality would be somewhat lower. However, in our series these litters commonly showed a poorer type of rat. They were most often produced by second rate animals or from the accidental matings of very young animals. Also, it must be considered that moribund young of larger litters of low vitality might have been eaten by the mother before being found and recorded, thus swelling the number of substandard small litters.

The number of very large litters (13 to 16 animals) is too small to warrant definite conclusions being drawn. However, the chances for exposure and mechanical deaths are undoubtedly increased in these, and it is not unlikely that they would show a higher than average death rate even in a more

extended series. The young of the very large litters are almost always noticeably strong and hardy, so that if part of the litter were given to other mothers the death rate might be considerably reduced.

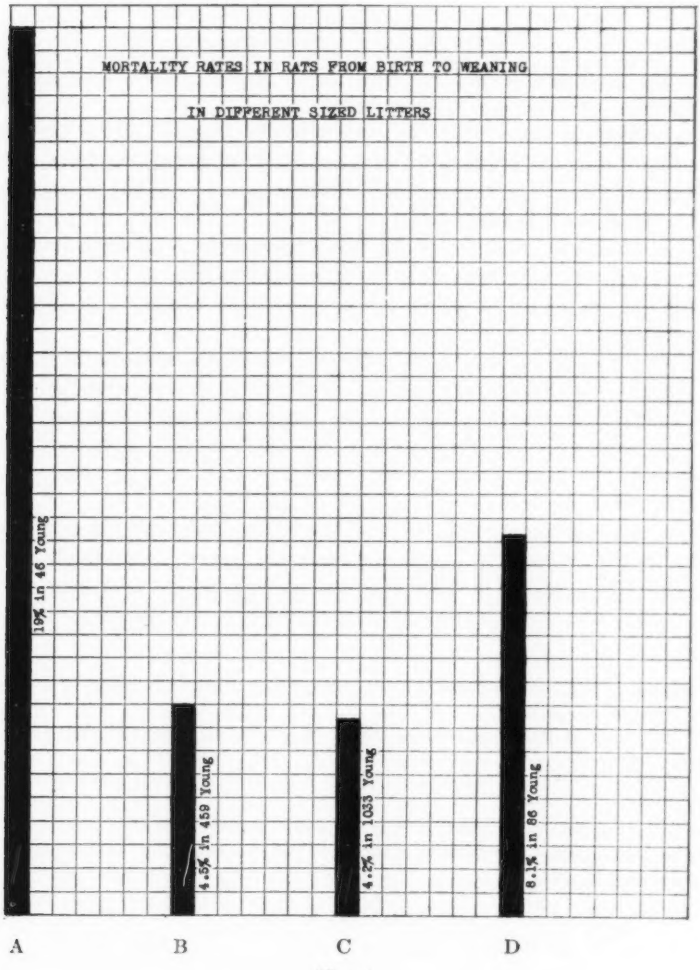

Fig. 1

A—Small: (1–4 young), 14 litters, 46 young
B—Medium: (5–8 young), 68 litters, 459 young
C—Large: (9–12 young), 101 litters, 1033 young
D—Very large: (13–16 young), 6 litters, 86 young

The "large" and "medium" litters had a distinctly lower death-rate than the "small" or the "very large" litters.

Litters of medium size (5 to 8 animals) showed a mortality of 4.5 per cent; large litters (9 to 12 animals), 4.2 per cent. These results clearly indicate that the viability of the young is not decreased by increasing the size of the nursing litter.

SUMMARY AND CONCLUSIONS

1. This investigation covered a period of five years and was made on 1,624 young born on a normal stock diet.

2. Consistently larger litters were produced by careful selective breeding.

3. The strain of large litters on lactating mothers, as evidenced by change in weight of the mother during the lactating period, was not greater than that in the case of smaller litters.

4. An increase in the size of the litter tended to be accompanied by a small decrease in the average birth weight of the young.

5. Weight gains up to weaning were essentially the same in large and small litters.

6. Weight gains up to weaning were essentially the same in large litters unreduced in size as in large litters reduced early in the lactation period.

7. In litters of from 9 to 12 young mortality during lactation was slightly but not appreciably lower than in litters of from 5 to 8 young. Very small and very large litters (1 to 4 and 13 to 16 animals respectively) showed higher death rates.

From these data it is concluded that the young from large litters compare favorably in hardiness and viability with the young from small litters, and that the increased number of littermates obtainable from large litters (up to 13 young per litter) may safely be used in controlled experimental work.

ADDENDA. For those not readily conversant with statistical methods it may be desirable to point out that

1. To be significant the difference between two means under consideration must be at least four times as great as the probable error of their difference.

2. To be significant a correlation coefficient must be at least four times its probable error.

3. If the population on which data are available is fully representative, i.e., if the distribution is normal, then the range divided by the standard deviation should yield a figure approaching six.

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STUDIES IN THE B VITAMINS

II. Statistical Comparison of Rat Litters on Normal Stock Diets with Litters on Synthetic Diets Containing Varying Amounts of the Vitamin B Complex and Combinations of B_1 and B_2

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The results of total deprivation of the vitamin B complex are so striking as to show sharply in small, critical experiments. The clear clinical symptoms and the rapid cures have become classic. When, however, the study of the vitamin B complex and its components is concerned not with total deprivation but with optimum and less than optimum amounts no sharp lines are discernible. Individual variations are marked. Apparently carefully duplicated experiments often give dissimilar results. Small numbers of animals become undependable.

The purpose of this study was to apply the statistical method of approach to a sufficiently large number of animals born on certain diets partially defective in all or part of the vitamin B complex, and to compare the results with those obtained with rats born on normal stock diets.

In the following paper a comparative study is made of females on stock and on the experimental diets under consideration, and our statistical data obtained during the lactation period on 2,627 young born on five of our diets are reported. These data include a, weight changes in mothers during lactation; b, size of litters; c, average birth weights; d, average weight gains to weaning, and e, mortality of the young during lactation. The diets include normal stock and four synthetic diets of which two supplied the vitamin B complex in the form of dried brewers yeast,[1] respectively 2 per cent and 10 per cent, and two supplied the B_1 and B_2 factors in varying proportions in the form of preparations made by this laboratory. These last two diets contained respectively 10 per cent B_1 plus 2 per cent B_2 and 5 per cent B_1 plus 10 per cent B_2.

A detailed account of our methods of securing the B_1 and B_2 products is given in paper III of this series (1), as is an explanation of the basis of calculating the percentages of the vitamin B factors in the diet.

[1] Northwestern Yeast Co.

Our B_1 (antineuritic, B, or F) factor was obtained by extracting yellow corn meal[2] with acid 85 per cent alcohol and adsorbing it onto corn dextrin. The B_2 (growth, P-P, or G) factor was made by autoclaving a 20 per cent suspension of yeast in tap water for 3 hours at 15 pounds pressure. For convenience we took 10 grams of basal food per animal per day as a standard from which to calculate percentages of the vitamin factors in the diet. Thus "10 per cent" is not an actual percentage but represents that one gram of the substance dry or in suspension, or the the extract or concentrate of one gram of that substance, was being supplied in measured amounts to each animal each day. All vitamin portions were carefully measured and fed to each animal before the basal food was placed in the cage.

Two years were spent in standardizing the methods of preparation of our B_1 and B_2 products and the percentages for feeding. Standardization was based on the following points. *a*. Amounts of the B_1 or B_2 preparations, separate or combined, necessary to effect cures in rats on diets deficient in these respective vitamin factors. Comparison of these amounts with curative quantities of whole yeast. *b*. Growth curves of animals on diets supplying B_1 or B_2 or combinations of these factors. *c*. Mortality on these diets as compared with that on yeast and on stock diets; and *d*, oestrous cycles of females on experimental and normal diets. Results of this standardization led us to believe that our B_1 product was relatively pure, but that the B_2 preparation probably contained small quantities of B_1 and of some other factor then unknown. The work of Reader (2) as compared to our findings indicates to us that this was probably B_4.

The stock diet consisted of McCollum's grain mixture using whole grain products, fresh minced vegetables, whole milk, and a small portion of table scraps. Cod liver oil[3] and iodine salts were supplied in addition. Experimental rats received casein 18, dextrin 78, salt mixture (no. 185 McCollum) 4, and iodine salts. Their vitamin portions, which were fed separately, consisted of 1 cc. of cod liver oil[3] per animal per day, 4 drops of wheat germ oil[3] (E) per animal per day, and the suitable amount of the B vitamin. The experimental diets varied only in the form and amounts of the vitamin B complex or its components supplied.

Stock rats were kept in large, sawdust-bottomed cages. Experimental animals had cages with wire mesh botoms. Females were placed in individual cages, segregated before delivery and given shredded paper for nest building. Stock males were used in all matings.

Since the work of this laboratory was primarily concerned with pregnancy and lactation and only secondarily with growth after weaning, many of the standard methods of vitamin determination have proved to be

[2] Quaker Oats Co.
[3] Mead, Johnson Co.

hardly satisfactory for our purposes. The growth curve of a rat transferred from stock to an experimental diet after weaning does not indicate the sexual maturity or potential fecundity of that individual animal. Nor does it show whether delivery and lactation of young are possible. We determined therefore to base our study entirely on young born of mothers raised on the diets in question.

The litters born on the antineuritic factor (B_1) and on the growth factor (B_2) are all first generation young, being first and second litters of mothers transferred from stock to the experimental diets at from two to three months of age. The earliest litters were obtained only after the mothers had been on the diets for over three months.

A study of the oestrous cycles of females on the stock and the experimental diets was made, using Evans' method (3) of microscopic examination of the vaginal smears. Oestrus occurred in our stock females regularly on the average of about every fourth to fifth day (4). The smears were definite and easy to identify, having large numbers of the characteristic oestrous cells predominating. On the experimental diets, however, particularly in those supplying the B_1 and B_2 preparations considerable variation was seen, both as concerned the length and regularity of the oestrous cycle as well as the type of cells occurring in the smear. It was frequently very difficult to determine when true oestrus was present. Typical oestrous cells were not always seen, and when present were usually smaller and more mixed among leucocytic and epithelial elements than when seen in stock vaginal smears. Frequently, nothing but masses of cellular debris was found. We have been led to believe that this may have represented an abnormal oestrous period.

An examination of our smear records showed in the case of animals on the 10 per cent B_1 2 per cent B_2 diet that at the end of one month on the diet the average oestrous cycle was from 5 to 6 days; at the end of six months, from 7 to 8 days. Individual cycles were very irregular. On the 5 per cent B_1 10 per cent B_2 at the end of one month the average oestrous cycle was 5 to 6 days; at the end of six months, 6 to 7 days. These cycles were also irregular. On 2 per cent yeast, after several months on the diet, average oestrous cycles were 6 to 7 days, while on the 10 per cent yeast diets at this time oestrous cycles were approximately normal, being 4 to 5 days but more irregular than in our stock animals.

Our available data on the diets concerning weight changes in mothers during the lactation period are summarized in table 1. The number of females on the B_1 plus B_2 diets producing young which survived the four weeks of nursing was so small that data on their weight changes are not dependable in a statistical study. This is more or less true of the yeast diets, but it seems worth reporting that so far as they may be relied upon they indicate no greater strain on lactating females on two per cent or ten per cent yeast diets than on the normal stock diet.

A comparative study of litter size, given in table 2, shows that the stock litters were significantly larger in number of young than those of any of the synthetic diets with vitamin B added. The diet quality when judged by this standard shows the following order of value, *a*, stock; *b*, 10 per cent yeast; *c*, 5 per cent B_1 10 per cent B_2; *d*, 10 per cent B_1 2 per cent B_2; *e*, 2 per cent yeast. However, the differences in litter size among the experimental diets are not large enough to be statistically significant.

Table 3 indicates the comparison of average birth weights. The stock weights are significantly higher than those on any of the experimental

TABLE 1 *

Weight changes in mothers during lactation

DIET	NUMBER OF MOTHERS	WEIGHT CHANGES RANGE	MEAN WEIGHT CHANGE	PROBABLE ERROR OF MEAN	STANDARD DEVIATION	RANGE / STANDARD DEVIATION	DIFFERENCE OF MEANS / PROBABLE ERROR$_{dif.}$
One week:							
Stock	72	−40–+35	+0.07	±1.07	±13.50	5.36	
2 per cent yeast	23	−30–+20	−2.17	±1.62	±11.78	4.24	0.66
10 per cent yeast	22	−25–+30	−0.23	±1.87	±13.01	4.61	0.05
Two weeks:							
Stock	68	−50–+25	−4.52	±1.59	±19.38	3.87	
2 per cent yeast	19	−40–+25	−0.79	±2.38	±15.36	4.23	0.88
10 per cent yeast	19	−25–+50	−3.42	±2.43	±16.87	4.74	0.26
Three weeks:							
Stock	63	−50–+30	−5.63	±2.17	±20.33	3.94	
2 per cent yeast	20	−50–+25	−7.25	±2.68	±17.79	4.22	0.32
10 per cent yeast	16	−35–+30	−1.25	±2.35	±16.47	4.26	1.14
Four weeks:							
Stock	51	−65–+50	−4.39	±2.15	±22.76	5.05	
2 per cent yeast	15	−40–+30	−8.00	±3.16	±18.15	3.86	0.64
10 per cent yeast	14	−60–+40	−8.93	±3.78	±26.31	3.99	0.70

With the small numbers of animals compared, no significant difference was demonstrated in weight changes in mothers during lactation between diets containing yeast as a sole source of vitamin B and stock diet.

diets. The order of highest average weights proved to be *a*, stock; *b*, 10 per cent yeast; *c*, 5 per cent B_1 10 per cent B_2; *d*, 2 per cent yeast; *e*, 10 per cent B_1 2 per cent B_2. Among the experimental diets the differences are not large enough to be statistically significant.

A study of the average individual gains in weight during the lactation period on the different diets (table 4) shows that only the 2 per cent yeast diet is significantly below that of the stock at any time. By weaning, at the end of the fourth week, weight gains on 10 per cent yeast, stock, and 5 per cent B_1 10 per cent B_2 are found to be practically on a par with each other. Ten per cent B_1 2 per cent B_2 shows a definitely though not sig-

nificantly lower mean, but 2 per cent yeast maintains its consistently low position. It must be considered that all of the experimental diets have

TABLE 2

Comparative size of litters

DIET	NUMBER OF LITTERS	NUMBER OF YOUNG	RANGE IN NUMBER OF YOUNG	MEAN NUMBER OF YOUNG	PROBABLE ERROR OF MEAN	STANDARD DEVIATION	RANGE / STANDARD DEVIATION	DIFFERENCE OF MEANS / PROBABLE ERROR$_{dif.}$
Stock (total)	189	1,624	2–16	8.59	±0.13	±2.59	5.8	
Stock (1928–1929 only)	72	667	2–14	9.26	±0.20	±2.50	5.2	
2 per cent yeast	40	226	1–12	5.65	±0.29	±2.73	4.8	6.3
10 per cent yeast (total)	57	379	1–11	6.61	±0.19	±2.12	5.2	5.9
(1929 only)	22	161	3–10	7.23	±0.25	±1.76	4.5	4.3
10 per cent B_1 2 per cent B_2 (1929)	17	120	2–9	6.64	±0.28	±1.71	4.7	5.1
5 per cent B_1 10 per cent B_2 (1929)	24	172	5–12	7.17	±0.26	±1.89	4.2	4.4

This table shows that stock animals produced significantly more young per litter than rats on any of the synthetic diets.

Our experiments with 10 per cent B_1 plus 2 per cent B_2 and 5 per cent B_1 plus 10 per cent B_2 diets were carried on in 1929 only. An increase in litter size produced (by selective breeding) at that time has been reported in paper I of this series (4). For purposes of comparison, therefore, stock for 1928 and 1929 and 10 per cent yeast litters for 1929 only are given.

TABLE 3

Average weight of young at birth

DIET	NUMBER OF LITTERS	NUMBER OF YOUNG	WEIGHT RANGE	MEAN WEIGHT	PROBABLE ERROR OF MEAN	STANDARD DEVIATION	RANGE / STANDARD DEVIATION	DIFFERENCE OF MEANS / PROBABLE ERROR$_{dif.}$
			grams	*grams*				
Stock	164	1,379	3.7–6.5	5.27	±0.03	±0.58	5.0	
2 per cent yeast	33	189	3.0–6.0	4.49	±0.06	±0.55	5.5	7.1
10 per cent yeast	54	371	3.0–6.2	4.79	±0.05	±0.60	5.3	5.3
10 per cent B_1 + 2 per cent B_2	13	86	3.3–5.1	4.37	±0.11	±0.57	3.3	5.3
5 per cent B_1 + 10 per cent B_2	18	135	3.5–5.4	4.64	±0.08	±0.50	3.8	4.8

The mean stock weight is significantly higher than the means of any of the experimental diets.

percentage mortalities almost double those of the stock, and that it is usually the smaller rats that die, thus tending to raise the weight averages.

(However, from 75 per cent to 90 per cent of deaths on all diets come during the first week.) Later growths on the B_1 plus B_2 diets will be reported in paper III of this series (1).

The mortality of young during the lactation period is given in table 5.

TABLE 4

Weight gains by week to weaning

DIET	NUMBER OF LITTERS	NUMBER OF YOUNG	WEIGHT GAIN RANGE	MEAN WEIGHT GAIN	PROBABLE ERROR OF MEAN	STANDARD DEVIATION	RANGE / STANDARD DEVIATION	DIFFERENCE OF MEANS / PROBABLE ERROR$_{dif.}$
			grams	*grams*				
One week:								
Stock	91	708	1–12	6.20	±0.14	±1.99	6.01	
2 per cent yeast	22	123	0–9	4.86	±0.27	±1.87	5.35	5.35
10 per cent yeast	33	193	2–10	5.49	±0.28	±2.37	3.79	2.28
10 per cent B_1 + 2 per cent B_2	9	63	4–9	6.00	±0.38	±1.70	3.53	0.49
5 per cent B_1 + 10 per cent B_2	18	111	2–10	5.94	±0.30	±1.90	4.74	0.76
Two weeks:								
Stock	87	683	4–26	13.63	±0.27	±3.73	6.17	
2 per cent yeast	20	113	6–15	10.50	±0.46	±3.02	3.31	5.92
10 per cent yeast	26	131	7–22	13.50	±0.54	±4.08	3.92	0.22
10 per cent B_1 + 2 per cent B_2	9	63	5–28	13.67	±1.05	±4.67	5.14	−0.07
5 per cent B_1 + 10 per cent B_2	18	111	5–19	12.56	±0.60	±3.79	3.96	1.63
Three weeks:								
Stock	79	595	10–40	22.16	±0.43	±5.72	5.25	
2 per cent yeast	17	103	8–25	15.29	±0.78	±4.79	3.76	7.50
10 per cent yeast	23	107	14–34	20.09	±0.74	±5.25	4.00	1.24
10 per cent B_1 + 2 per cent B_2	8	56	15–30	21.13	±1.06	±4.43	3.61	2.32
5 per cent B_1 + 10 per cent B_2	14	87	8–30	20.00	±1.14	±6.35	3.62	1.77
Four weeks:								
Stock	66	509	15–59	31.72	±0.87	±10.44	4.31	
2 per cent yeast	13	72	13–36	23.92	±1.32	±7.06	3.40	4.94
10 per cent yeast	20	89	20–56	32.75	±1.42	±9.40	3.85	−0.61
10 per cent B_1 + 2 per cent B_2	7	41	18–38	28.71	±1.14	±4.46	4.71	2.10
5 per cent B_1 + 10 per cent B_2	10	66	20–40	31.00	±1.30	±6.08	3.45	0.46

Comparison of the weight gains of the young during the lactation period demonstrates a significantly lower gain for the 2 per cent yeast diet than for stock for the four weeks as well as for the shorter periods. The other experimental diets show no significant variation from stock.

The most striking point to be noted is the high death rate on all experimental diets. Since our stock young were raised in sawdust-bottomed cages and the experimental young in wire-bottomed cages, the possibility of exposure being an influencing factor in the mortality of the latter was considered. Fifteen litters on the experimental diets were born and kept in the regulation stock cages. This group showed a somewhat, although not strikingly lower mortality than was usual for the diets. On the other hand, over 100 stock young born and kept in the wire-bottomed cages showed no increase in death rate.

TABLE 5

Mortality during lactation

DIETS	NUMBER OF LITTERS	NUMBER OF YOUNG	PER CENT OF TOTAL DEATHS DURING LACTATION								TOTAL MORTALITY	
			Mortality first week		Mortality second week		Mortality third week		Mortality fourth week			
			Number	Per cent	Number	Per cent	Number	Per cent	Number	Per cent	Number	Per cent
Stock	189	1,624	66	82.5	4	5.0	7	8.8	3	3.8	80	4.98
2 per cent yeast	39	327	103	74.1	2	1.4	21	15.1	13	9.3	139	61.2
10 per cent yeast	57	380	177	85.1	15	7.2	7	3.4	9	4.3	208	54.7
10 per cent B_1 + 2 per cent B_2	19	126	57	90.5			3	4.8	3	4.8	63	50.0
5 per cent B_1 + 10 per cent B_2	24	172	54	85.7			2	3.2	7	11.1	63	36.8

Consistently high mortalities among the young (36.8 to 61.2 per cent) during the lactation period were found on all of the experimental diets. Mothers on a stock diet lost only 4.98 per cent of their litters.

SUMMARY

1. Statistical studies are reported on 1,624 normal stock young and on 1,003 young born on experimental diets.

2. A comparison of the oestrous cycles in the mothers of these young is made. Among the experimental diets only on 10 per cent yeast does the oestrous cycle remain approximately normal.

3. Stock litters and the litters born on four synthetic diets varying only in vitamin B content are compared.

4. No significantly greater weight changes on the part of the mothers was found in females nursing litters on a 2 per cent and a 10 per cent yeast diet than in stock females.

5. Litter size (mean number of young per litter) was found to decrease progressively in the following order: stock, 10 per cent yeast, 5 per cent B_1 10 per cent B_2, 10 per cent B_1 2 per cent B_2, 2 per cent yeast. There was

a significant difference between stock and all the experimental diets. Among the experimental diets, no significant difference was found.

6. The mean average birth weights were found to be progressively lower in the following order: stock, 10 per cent yeast, 5 per cent B_1 10 per cent B_2, 2 per cent yeast, 10 per cent B_1 2 per cent B_2. There was a significant difference between the stock and all the experimental diets. Among the experimental diets, no significant difference was found.

7. The comparative average individual weight gains during the lactation period showed considerable variation, with 2 per cent yeast always significantly lower than stock. At weaning (28 days) 10 per cent yeast, stock, and 5 per cent B_1 10 per cent B_2 were practically equal, with 10 per cent B_1 2 per cent B_2 considerably but not significantly lower.

8. Very high mortality rates among the young during the first four weeks of life were found on all experimental series. Possibility of exposure in the wire cages being an influencing factor is considered.

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STUDIES IN THE B VITAMINS

III. Evidence of a Third Vitamin B Factor in Yeast (B_4) as Shown by Growth Curves and Clinical Symptoms of First and Second Litter Young of Mothers Raised on Synthetic B_1 and B_2 Diets

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In attempts to analyze the clinical effects of the B_1 and B_2 factors of the vitamin B complex, this laboratory has considered it advisable to use, if possible, animals other than those just weaned from stock diets. By so doing we hoped to eliminate such effects as might be associated with a possible storage of vitamin factors supplied to our normal stock animals (1). It was desirable to secure such synthetic diets, supplying the B_1 and B_2 factors, as would permit our experimental animals to mature, reproduce, and nurse their young.

Since our main interest was to study clinical effects, we sought in our preparing of the B_1 and B_2 factors to establish standardized methods of securing products which, though not necessarily pure in the respective vitamin factors, would be of high enough concentration to maintain our animals approximately normally for several months.

At the time this series of experiments was started (1929) our B_1 and B_2 preparations had been carefully standardized. Certain discrepancies in our findings and the work of others (2) had led us to suspect the presence in yeast of at least one other factor of the vitamin B complex. In our preliminary work on the preparation of a suitable B_2 product (1928) using neutral suspensions of yeast[1] autoclaved over periods varying from 1 to 10 hours at 15 pounds pressure, we found that autoclaving for more than three hours gave us products which were increasingly less satisfactory for the maintenance of our experimental animals to maturity. We therefore adopted as our B_2 preparation a 20 per cent suspension of yeast in tap water autoclaved for 3 hours at 15 pounds pressure, stored in refrigeration until used. (A two week's supply was autoclaved at one time.) Our standardization of the potency of this product led us to believe that it

[1] Northwestern Yeast Co.

probably contained small amounts of the B_1 factor and also of some other factor then unknown, but that it was eminently satisfactory for our purpose of maintaining animals to maturity on synthetic B_1 and B_2 diets. (The work of Reader (3) indicates to us that we were probably dealing with B_4.) A carefully standardized routine of preparation was established in order to maintain the amounts of B_2 as well as of the unknown substances in our product always at a constant level.

As a source of B_1 we chose an extract of yellow corn meal[2] made by macerating the meal with 85 per cent alcohol with 1 per cent HCl for one week, pressing out the fluid with a fruit press, washing with fresh acid 85 per cent alcohol, and filtering the mixed alcohols to free them from small corn meal particles. Most of the alcohol was evaporated off by heating 125 cc. portions for 15 minutes in a boiling bath, using a suction condenser apparatus. Small quantities of the fluid were taken to avoid subjecting the vitamin factor to prolonged heating. The concentrated extract was chilled, the protein precipitated by bringing the solution approximately to the iso-electric point by stirring in dilute NaOH, and filtered. The filtrate was adsorbed on white corn dextrin and dried by electric fan at room temperature. The resultant material, of which 1 gram was equivalent to 5 grams corn meal, was powdered, stored in a cool place, and fed daily in accurately measured amounts. This concentrate appeared to be relatively free of the B_2 factor. We had no indications that another factor might be present.

The food of our synthetic diets consisted of casein 18, dextrin 78, salt mixture (no. 185 McCollum) 4, and iodine salts. In addition, each of the experimental animals received as a daily portion 1 cc. cod liver oil[3] (vitamins A and D) and 4 drops wheat germ oil (vitamin E). The entire vitamin portion, including adequate, measured amounts of the B_1 and B_2 preparations, depending upon the diet, was carefully measured out and served in a small dish before the basal food was placed in the cage. In this way it was possible accurately to control the vitamin intake of each animal. The diets of the different experimental groups varied only in the amounts of B_1 and B_2 supplied.

It had been determined in our laboratory that approximately 10 grams of basal food per animal per day was a generous allowance, and for the sake of brevity and clearness this amount was adopted as a standard on which to calculate diets. Thus "10 per cent" of any substance is not an actual percentage in the diet, but means that 1 gram of that substance, dry or in suspension, or the extract or concentrate of 1 gram of that substance was being supplied to each animal each day. (In the case of our antineuritic concentrate we used as 10 per cent the extract of 1.2 gram of corn meal to allow for possible loss of the B_1 factor during preparation of the concentrate.)

[2] Quaker Oats yellow corn meal.

[3] Mead, Johnson Co.

On May 1, 1929, two new experimental diets, CXIX and CXX were started. The diets were as given above, with the addition of: in group CXIX, 10 per cent B_1 2 per cent B_2 (0.25 gm. corn meal concentrate and 1 cc. 20 per cent suspension autoclaved yeast); and in group CXX, 5 per cent B_1 10 per cent B_2 (0.13 gm. corn meal concentrate and 5 cc. 20 per cent suspension autoclaved yeast). Hereafter these diets will be referred to simply as CXIX and CXX respectively. A group of 12 normal stock females ranging in age from 70 to 90 days was placed on each of these diets. They appeared to develop normally.

The vitamin proportions here used were based on results of our preliminary work in which it appeared that 10 per cent B_1 or 10 per cent B_2 were the maximum quantities necessary for optimum development of experimental animals taken from stock. These amounts had proved to be effective dosages for the treatment of animals suffering from a deficiency of one or the other vitamin factors.

The animals were kept in large screen-bottomed cages (4 meshes per inch). They continued to gain weight and appeared in excellent condition throughout the entire experiment.

After more than five months on these diets some of the females were impregnated by stock males and litters were produced. After the young were weaned, the mothers were given the usual resting period of one month, and then attempts were made to remate them. Completely failing this, the animals from both groups were deprived of the vitamin B_1 and B_2 factors and instead for one month were given 2 per cent yeast as a sole source of vitamin B. They were then returned to their respective B_1 and B_2 diets. At the end of this time successful matings with stock males, were secured in five of the seven original mothers from each group. At this time the females had been on the respective diets for eight months (with the exception of the one month on 2 per cent yeast).

Comparison of first and second litters. The birth and lactation reports of these young have been presented in paper II of this series (3). Because of the similarity in all findings up to weaning, first and second litters are there grouped together (with other young born on the same diets). In that paper it was indicated that on such diets, as compared with stock, were obtained 1, fewer young per litter; 2, smaller birth weights, and 3, a high mortality. However, weight gains for the surviving young during the lactation period were the same as for our normal stock animals in the case of diet CXX and not significantly lower for that of CXIX.

Compared with each other, no difference of any significance was obtained between first and second litters in number of young per litter, average birth weights, average weight gains to weaning, or mortality.

Experimental groups using first litter young. After weaning the first litters, the young from each of the original diets were segregated into

groups to investigate the following points: 1, the effect of continuing the offspring on the original diets of their mothers, respectively CXIX and CXX; 2, the effect of withdrawing the factor which had been supplied in the lesser proportion in the original diet, leaving respectively only *a*, 10 per cent B_1, and *b*, 10 per cent B_2; 3, the effect of withdrawing both factors, viz., reduction to a vitamin B free diet; 4 the effect of the substitution of 2 per cent yeast in place of the B_1 and B_2 preparations; 5, the effect of supplying 10 per cent of each of the B_1 and B_2 factors. On the twenty-eighth day of the experiment, two animals from each group (5) above were transferred to ascertain (5′) the effects of supplying 20 per cent of each of the B_1 and B_2 factors.

The vitamin B portions of the diets then varied as follows;

Young from CXIX mothers	*Number of rats*	*Young from CXX mothers*	*Number of rats*
1. 10 per cent B_1 2 per cent B_2.	4	1. 5 per cent B_1 10 per cent B_2.	4
2. 10 per cent B_1 No B_2.....	6	2. No B_1 10 per cent B_2.....	5
3. No vitamin B.....	5	3. No vitamin B.....	5
4. 2 per cent yeast.....	5	4. 2 per cent yeast.....	4
5. 10 per cent B_1 10 per cent B_2.	6	5. 10 per cent B_1 10 per cent B_2.	4
5′. 20 per cent B_1 20 per cent B_2.	(2)	5′. 20 per cent B_1 20 per cent B_2.	(2)
Total.....	26	Total.....	22

First litter groups. Numbers of males and females were approximately equal and were evenly distributed among the groups. This series was continued for a period of sixty days. The average growth curves are seen in the accompanying chart.

Experimental groups using second litter young. After weaning the second litter young, 16 on the CXIX diet and 15 on the CXX diet were available for this experiment. After consideration of the findings from the first group of experiments, it was decided not to repeat all of those tests. Instead, the second litter young were divided into four groups in the following order (males and females again being evenly distributed):

Group 1. Six animals from CXX mothers, placed on a diet containing 20 per cent B_2 no B_1.

Group 2. Five animals from CXX mothers, placed on a diet containing 15 per cent B_1 20 per cent B_2.

Group 3. Six animals from CXIX mothers and 5 animals from CXX mothers, placed on a diet containing 20 per cent B_1 20 per cent B_2.

Group 4. Nine animals from CXIX mothers, placed on a diet containing 50 per cent B_1 20 per cent B_2.

(Please note again that the vitamin rations are not actual percentages, but indicate amounts based on 10 grams basal food per day per animal as 100 per cent. Thus "50 per cent B_1" means that 1.25 gram of the corn meal concentrate was fed each animal daily.)

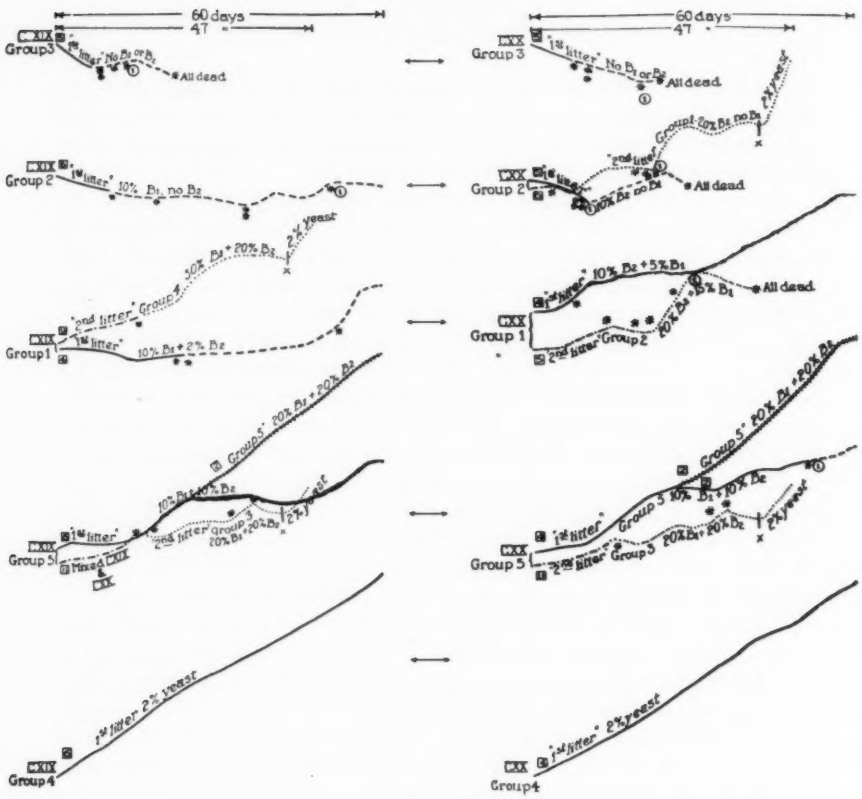
It was hoped in this grouping to enhance such effects as might be associated with predominance of either factor in the mothers' diets. This series was continued for 42 days, after which for 5 days 2 per cent yeast was substituted in place of the B_1 and B_2 factors.

Arrangement of growth curves. The resultant average growth curve for each group is given on the chart, the second litter group accompanying that of the first litter group in which the heredity and diet are most comparable. Thus second litter group 1 is compared with first litter CXX-2, since the young in both cases were from CXX mothers and the diets supplied no B_1. Second litter group 2 is compared with first litter CXX-1, since again the mothers were from diet CXX and the group diets supplied both B_1 and B_2 factors with the latter in the greater proportion. Second litter group 3 is compared to the 5 and 5′ groups from both CXIX and CXX first litter groups, since both maternal diets are represented, and B_1 and B_2 are supplied in equal proportions. Second litter group 4 is compared with first litter CXIX-1, since the maternal diet was CXIX in each case, and also the diet of the young contained both B_1 and B_2 with the B_1 predominating. The last curve may be compared also to CXIX-5′, since both the B_1 and B_2 factors were supplied in relatively large amounts.

It will be noted in the following discussions that there is considerable variation in the response of individual animals to certain diet deficiencies. The majority of a diet group may die very early, while a single individual of the same heredity may survive and even show some gain in weight. It was impossible in this experiment to compensate for such a factor of error by the use of large numbers of animals. While it is of importance to consider the animal which acts atypically, we feel that the behavior of the majority of animals in the group should be chosen as the standard for comparison. Apparent gains in weight, due to deaths of smaller animals, should be proportionately discounted on this basis.

Analysis of first litter growth curves with clinical symptoms. Group CXIX-1: Four rats from CXIX mothers continued on the 10 per cent B_1 2 per cent B_2 showed little gain during the 60 day period, the occasional slight rises in the curve usually being associated with the deaths of the smaller animals. One death occurred on each of the twenty-first, twenty-second, and fifty-second days. (Only one animal remained alive throughout the experiment.) On this diet the animals showed an increasingly unkempt condition, the hair becoming ragged, greasy, and yellow. They were generally inactive and walked humped up, on their toes, and with a rolling gait as their condition became worse.

Group CXX-1: Four animals from CXX mothers continued on the 5 per cent B_1 10 per cent B_2. (On the fifteenth day one of these escaped from the cage and was discarded from the experiment.) They showed a small but steady gain. The only clinical symptoms noted on this diet were an



CODE

□ Number of animals (mixed ♀ and ♂) started on group.
* Death of one animal.
——— All animals in group alive (1st litter young).
---------- After first death (1st litter young).
-·-·-·-·- All animals in group alive (2nd litter young).
..... After first death (2nd litter young).
++++++++ 2 animals transferred to 20 per cent B_1 + 20 per cent B_2 on 28th day.
① Only one animal remaining.
× Changed to 2 per cent yeast on 42nd day.

Fig. 1. Growth curves comparing first and second litter young born of mothers that were reared from weaning time on synthetic diets containing varying amounts of B_1 and B_2.

The basal diet consisted of casein 18, dextrin 78, salt mixture 4 and iodine salts with 1 cc. cod liver oil and 4 drops wheat germ oil plus measured amounts of yeast (groups 4) or of extracts of yeast and corn meal for the B_1 and B_2 factors (groups 1, 2 and 5). Groups 4 on 2 per cent yeast show approximately normal growth curves. The other curves indicate lack of growth and subsequent death (groups 2 and 3) or varying degrees of growth (groups 1 and 5) in proportion to the amounts of B_1 and B_2 supplied.

occasional tendency to walk on the toes and a greasiness of the hair which appeared intermittently. There were no deaths.

Group CXIX-2: Six rats from CXIX mothers were continued on 10 per cent B_1 but no B_2 was given. The weight curve shows a small loss with occasional apparent slight gains thereafter as the smaller animals died. Deaths occurred on the eighteenth, the thirty-fourth, and thirty-fifth, and the forty-ninth days. (One animal lived throughout the experiment.) The clinical symptoms developed during the period were greasiness, yellowing, and thinning of hair, nasal hemorrhage, an abnormal rolling gait, and general evidences of inanition.

One rat was transferred to a curative diet of 20 per cent B_1 20 per cent B_2 on the tenth day. His curve thereafter showed a rapid and approximately normal gain to the sixtieth day.

(Note: animals removed from the diets for "cure" are counted as deaths on the growth curves, as experience has shown us that death rapidly follows the appearance of deficiency symptoms unless a curative diet is supplied. Weight curves kept on these cure animals are not given in the chart.)

Group CXX-2: Five rats from CXX mothers were continued on 10 per cent B_2 but no B_1 was given. The weight curve shows a steady drop followed by an apparent rise due to the deaths of the smaller animals. Three deaths occurred on the eighth and ninth days and one on the twenty-sixth day. The clinical symptoms presented were very similar to those in CXIX-2 above except that spastic paralysis developed in two of these rats before death.

One rat was transferred to the curative diet of 20 per cent B_1 20 per cent B_2 on the tenth day. This animal was apparently moribund and had to be fed by pipette for several days. After about a week on the cure diet and although improving gradually from his severe state of inanition, he developed a partial paralysis which lasted for almost a week. The same vitamin treatment was continued and he gradually improved, finally beginning to gain normally after about twenty-five days on the cure. After two weeks of normal weight gain another plateau in his curve appeared.

Group CXIX-3: Five rats from CXIX mothers were fed no vitamin B in any form. The weight curve shows an immediate loss in weight and early deaths. Deaths occurred on the eighth, eleventh, and twentieth days. The clinical symptoms were a general unkempt condition, hunched posture, and rolling gait, inanition, nasal hemorrhage, and in two cases a spastic paralysis.

One rat transferred to 20 per cent B_1 20 per cent B_2 on the tenth day showed a steady although slightly subnormal gain.

Group CXX-3: Five rats from CXX mothers fed no vitamin B in any form showed an immediate loss in weight and early deaths. These occurred on the eighth, tenth, twentieth, and twenty-first days. The

clinical symptoms were the same as for group CXIX-3 above with the two cases of spastic paralysis.

One rat transferred to 20 per cent B_1 20 per cent B_2 on the tenth day showed immediate improvement and better than average normal gain to the end of the experiment.

CXIX-4 and CXX-4: Five rats from CXIX mothers were placed on CXIX-4 and four from CXX mothers on CXX-4. All received 2 per cent desiccated yeast as a sole source of vitamin B, this being the minimum amount usually necessary to produce normal growth after weaning. The growth in both groups was steady, equal, and normal as compared to stock animals of the same age. The rats were in excellent condition throughout, alert, active, and well-groomed. There were no deaths.

Groups CXIX-5 and CXX-5: Six rats from CXIX mothers were placed on CXIX-5 and four from CXX mothers on CXX-5, all receiving 10 per cent B_1 10 per cent B_2. These animals, although appearing to be in excellent condition and keeping alert and active, gained slowly. On the twenty-eighth day two rats from each group were transferred to CXIX-5′ and CXX-5′ respectively (vide infra). The rats remaining on the 10 per cent B_1 10 per cent B_2 diet continued to gain slowly. (One of the animals from CXIX-5 was accidently dropped and killed on the eighteenth day.) One of the animals from CXX-5, the smallest rat, showed almost no gain over a long period of time and died on the fifty-first day showing some spasticity but no characteristic paralysis.

Groups CXIX-5′ and CXX-5′: On the twenty-eighth day two rats from CXIX-5 and two rats from CXX-5 were transferred from the diet containing 10 per cent B_1 10 per cent B_2 to one containing 20 per cent B_1 20 per cent B_2. They showed an immediate improvement and a normal growth to the end of the experiment (60 days), being comparable in every way to the animals on 2 per cent yeast.

Interpretation of results on first litters: Young from CXIX and from CXX mothers showed no marked variation in response when transferred to the same diets (groups 3, 4, 5, and 5′). On 2 per cent yeast normal weight gains were obtained (groups 4). However, what had been a diet adequate to maintain the mothers (transferred from stock at two to three months) apparently normally for over five months, and which permitted gestation, delivery, and approximately normal lactation weight gains, proved inadequate for normal growth of the young after weaning (groups 1). More than double quantities of our respective B_1 and B_2 preparations were necessary to permit normal weight gains in these young (groups 5′).

The most typical clinical symptoms manifested by animals on the deficient diets (groups 1, 2, and 3) were hunched posture, abnormal rolling gait, greasy yellowed hair, and nasal hemorrhage. In some of the rats (6 cases) on B_1 deficient and B free diets (groups CXX-2, CXIX-3, and CXX-3) a terminal spastic paralysis was superimposed.

Analysis of second litter growth curves. 1. Six animals from CXX mothers placed on a diet containing 20 per cent B_2 but no B_1 showed a slight weight gain to the first death on the fourth day (graphed with CXX group 2). Subsequent deaths on the eighteenth and twenty-first days caused an apparent sharp gain due largely to the fact that only the largest animal in the group remained. (His curve showed some variation but practically no rise to the forty-second day when 2 per cent yeast was substituted for the B_2 in the diet. The response was then immediate, and the curve angulated sharply to a better than normal incidence.)

2. Five animals from CXX mothers placed on a diet containing 15 per cent B_1 20 per cent B_2 showed a slight gain up to the first death on the fifteenth day (graphed with CXX group 1). Subsequent deaths on the eighteenth, twenty-first, and twenty-fifth days caused a sudden apparent increase in weight. As in group 2 this was because only the largest animal remained, but this curve dropped steadily till death on the thirty-eighth day.

3. Six animals from CXIX mothers and five from CXX mothers placed in one group on a diet containing 20 per cent B_1 20 per cent B_2 showed a small gain with some variations, due largely to deaths on the fifteenth, thirty-second and thirty-fifth days. (Graphed with both CXIX and CXX group 5.) The sudden drop thereafter was abruptly reversed by the substitution of 2 per cent yeast for the vitamin B portion.

4. Nine animals from CXIX mothers placed on a diet containing 50 per cent B_1 20 per cent B_2 gained steadily, with one death on the fourteenth day, until the thirty-first day when the curve flattened out (graphed with CXIX group 1). After the thirty-fifth day it declined until sharply checked on the forty-second day by the substitution of 2 per cent yeast for the B_1 and B_2 rations.

Clinical appearance of second litter animals. The appearance of the second litter young in group 1, (no B_1 20 per cent B_2) and group 2 (15 per cent B_1 20 per cent B_2) was very similar. These animals were rather scrawny and showed the usual greasy, yellowed fur. Typical paralysis was not seen prior to death, although some spasticity did occur in a few cases. Usually the animals sat hunched up and remained apparently somnolent until they fell on their sides from weakness. In a number of cases red edematous swelling of the feet, particularly of the hind feet, occurred.

On group 3 (20 per cent B_1 20 per cent B_2) and group 4 (50 per cent B_1 20 per cent B_2) the appearance was somewhat better, although all of the animals showed some greasiness of fur. Animals dying on these groups did not show typical paralysis but presented rather a picture of inanition. Nasal hemorrhage was almost invariably present in all the animals dying on any of the second litter diets.

Interpretation of results on second litters. On *none* of these diets supplying large amounts of B_1 or B_2 or both was normal growth obtained. Two per cent yeast, supplied on the forty-second day, elicited better than normal gains in all surviving groups. Spastic paralysis was not manifested in any animal on these diets.

Comparison of first and second litter animals. Approximately double the maternal B_1 and B_2 rations were required for normal growth after weaning in first litter young, but this amount proved quite inadequate for growth after weaning in second litter young. Both first and second litters responded normally to 2 per cent yeast. Clinical manifestations of deficiency in first and second litter young were very similar, with the exception that spastic paralysis occurred in some of the first litter animals, while swollen, red feet were seen in part of the rats from the second litters.

SUMMARY AND DISCUSSION. At the time this investigation was being carried out we were at a loss to explain, on the basis of B_1 or B_2 deficiency, the discrepancy in results obtained. Our B_1 and B_2 products, while admittedly not absolutely pure, still contained such high concentrations of the respective vitamin factors as to produce diverse, distinctive clinical pictures in animals taken from stock diets. Cures were readily brought about by administration of adequate dosages of the appropriate vitamin preparation. The rigidly standardized technique of preparing our B_1 and B_2 products seemed to preclude possible noticeable variations in their potency or nature. Previous investigations had indicated to us that our B_2 product contained traces of B_1 and probably of some other factor which was present in yeast. Our B_1 product we had shown to be practically free from B_2. We had had no indications that another factor might be present.

We had come to consider that 10 per cent as a maximum ration of the B_1 or B_2 preparations permitted optimum development.

In the present study the mothers (transferred from stock at 2 and 3 months of age) appeared to develop normally on 10 per cent B_1 2 per cent B_2 and 5 per cent B_1 10 per cent B_2 diets. The first litter young of these mothers born after five months on the synthetic diets, showed approximately normal weight gains to weaning. After weaning, however, when continued on the maternal diets they showed very inadequate growth. Normal growth in these young was possible only when the B_1 and B_2 portions were approximately double those in the maternal diets or when 2 per cent yeast was supplied as a source of vitamin B.

Second litters, born after eight months, could not be obtained until the mothers had been supplied with 2 per cent yeast for one month prior to impregnation. During lactation these second litter young showed approximately normal weight gains. After weaning, when placed on B_1 and B_2 rations which had proved adequate for normal growth of first litter young,

these second litter animals made very inferior progress. However, they responded immediately to 2 per cent yeast.

The clinical symptoms occurring in the young of either first or second litters varied from the characteristic pictures of either B_1 or B_2 deficiency. In the first litters deaths occurred on diets previously considered as adequate, while in the second litters animals died on diets containing relatively very high percentages of the B_1 and B_2 preparations. We found hunched up posture, abnormal rolling gait, greasy yellowed fur, and nasal hemorrhage as a characteristic clinical picture. In six of the first litter animals a terminal spastic paralysis was superimposed on this. Of the second litter young, none showed terminal paralysis, although some spasticity occurred in a few cases. Moribund animals invariably presented the picture described above with the addition in some cases of red edematous swelling of the feet, usually appearing only in the hind feet.

Reader (3) describes the clinical picture of B_4 deficiency as contrasted to polyneuritis resulting from a lack of B_1. We were surprised to find that her report gives identically (with the exception of nasal hemorrhage) a description of the condition we had found in our animals.

It appears evident then that in this study our results are closely associated with a lack of B_4. Assuming this to be true, the following explanation of our findings appears logical.

Preliminary work with our B_2 preparation (4) led us to believe that it contained traces of some other vitamin factor present in yeast as well as small quantities of B_1, since on 10 per cent B_2 we could raise part of our animals to maturity. That this third vitamin factor was probably B_4 was indicated when Reader described a factor which was present in yeast and necessary for the continued growth of the rat. In the present study, the better growth obtained on 50 per cent B_1 20 per cent B_2 than on 20 per cent B_1 20 per cent B_2 seems to indicate that small quantities of B_4 were probably present also in our B_1 preparation.

Storage of B_4 in our stock animals, together with the quantities of B_4 supplied as impurities in our B_1 and B_2 products permitted normal development of mothers of litters used in this experiment. Lesser storage of B_4 in the first litter young was indicated by their inadequate weight gains when continued on maternal diets, and by the appearance of B_4 deficiency clinical symptoms.

Although themselves apparently normal, the mothers had to be "restocked" with some B_4 (by giving 2 per cent yeast) before second litter could be obtained. These young showed very much less storage of B_4 since they required very large quantities of our B_1 and B_2 preparations, but the amount of B_4 so supplied was insufficient to produce normal growth in any of the diets used. These young died on much more than adequate B_1 and B_2 rations, showing typical symptoms of B_4 deficiency. Young

from both litters responded immediately and with adequate weight gains when supplied with minimal amounts of yeast.

It would seem then, that our work, through lack of a third vitamin factor found in yeast, closely corroborates that of Reader on vitamin B_4.

CONCLUSIONS

In order to explain our findings we must assume:

1. The presence in yeast of a third vitamin B factor. We identify this with Reader's B_4.
2. That deficiency of this factor produces a characteristic clinical picture distinct from those of B_1 or B_2.
3. That this factor is necessary for continued growth in the rat.
4. That this factor is stored in progressively lesser amounts in *a*, mothers from stock placed on synthetic diets; *b*, first litter young, and *c*, second litter young of these mothers.
5. That more of this factor is required for the production of litters than is necessary for the maintenance of adult females.
6. That this factor is present in mothers' milk in amounts adequate to produce normal weight gains in the young to weaning, even when the maternal diet is relatively deficient in this accessory.

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STUDIES IN THE B VITAMINS

IV. A Report of Litters Obtained on a Diet in Which Feces were Supplied as a Sole Source of Vitamin B

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The possible importance of the vitamin B content of feces as a factor influencing results obtained with rats on diets low in this food accessory was pointed out by Steenbock, Sell and Nelson (1). They found that growth was doubled when such rats had access to their excreta. McCollum, Simmonds and Becker (2) questioned these findings on the basis of possible errors of method and reported very comparable results on rats with and without access to their feces. Salmon (3) in a carefully conducted series of experiments using fresh, old, and fungus-grown feces corroborated Steenbock's findings. He indicated that even with wire-bottomed cages a certain error was possible, though of probable slight importance, because of the great avidity with which rats on a low-B diet would seize and devour the droppings.

We wished to determine *a*, if feces in the diet as a sole source of vitamin B were adequate to permit growth to maturity of animals born of mothers raised on low vitamin B diets; *b*, if litters could be secured from such animals.

Survival on feces diet. On the test experiment, 10 adult stock males were transferred from the stock diet to one in which 0.1 gram of dried brewer's yeast per animal per day was supplied as a sole source of vitamin B. These animals were kept in screen-bottomed cages, two meshes to the inch, and the droppings were collected daily. These feces, throughout the course of the experiment, formed the sole source of vitamin B in the diet of the group described below.

Experimental group: 25 animals mixed males and females, 2 to 3 months of age, born and weaned by mothers raised on low vitamin B diets[1] were placed in wire-bottomed cages (four meshes to the inch) and supplied our usual basal diet, and cod liver oil, wheat germ oil, and the fresh feces of the group of males mentioned above as a sole source of vitamin B.

Control groups: Our controls were made up of four groups of animals transferred from their original diets to a vitamin B free diet as indicated.

Group 1. Nine adult rats transferred from stock.
Group 2. Eight partly matured rats transferred from stock.
Group 3. Five partly matured rats transferred from diet CXIX.[1]
Group 4. Five partly matured rats transferred from diet CXX.[1]

All of these animals were kept in wire-bottomed cages and did not have access to their feces.

Summary of time of death on B-free diet

	DEATHS										
	1 week	2 weeks	3 weeks	4 weeks	5 weeks	6 weeks	7 weeks	8 weeks	9 weeks	10 weeks	11 weeks
Group 1. (9 adults from stock)							1	2	6	All dead	
Group 2. (8 young from stock)†						1		(1*)		2(3*)	1
Group 3. (5 young from CXIX)		4		1	All dead						
Group 4. (5 young from CXX)		3		2	All dead						

* Rats showing deficiency symptoms, cured by administration of adequate quantities of yeast. All the animals in this control experiment died as shown except the four that were given yeast.

† Four dead and four cured in this group.

Of the 25 animals on the test experiment (of the same parentage and diet as groups 3 and 4 above) receiving the same diet as the control groups with the exception that feces were added, 19 were still alive at the end of 8 months. At 10 months nine were living. The animals had now reached the age limit (12 months) set by this laboratory in all investigations of pregnancy or lactation, so the experiment was discontinued.

Litters on feces diet. The possibility of procuring litters on this very poor diet was of particular interest to us. The 25 rats on the feces diet described above were used. At first the males and females were segregated and attempts made to mate the females with stock males, placing the pairs together overnight. This being unsuccessful, the males and females on the feces diet were mixed. During several months eleven litters totaling 46 young were obtained (one female produced two litters).

We wish to emphasize that both parents had been raised on the "feces" diet.

For control, 8 stock females were impregnated and transferred immedi-

[1] CXIX and CXX, respectively 10 per cent B_1 + 2 per cent B_2, and 5 per cent B_1 + 10 per cent B_2, described in paper III of this series (4).

ately to a vitamin B-free diet. At the end of gestation 58 young were delivered.

In the accompanying tables (1, 2 and 3) are given the statistical results of our data on stock litters as compared with litters obtained on the feces diet, and the litters from mothers transferred from stock to a vitamin B-free diet when pregnant. The tables compare the number of young per

TABLE 1

Number of young per litter

DIET	NUMBER OF LITTERS	NUMBER OF YOUNG	RANGE IN NUMBER OF YOUNG	MEAN NUMBER OF YOUNG	PROBABLE ERROR OF MEAN	STANDARD DEVIATION	RANGE / STANDARD DEVIATION	DIFFERENCE OF MEANS / PROBABLE ERROR dif.
Stock	189	1,624	2–16	8.59	±0.13	±2.59	5.8	
B-free (when pregnant)	8	58	4–10	7.25	±0.52	±2.16	3.2	2.5
Feces	11	46	2–9	4.00	±0.42	±2.05	3.9	7.7

Rats depending on feces for vitamin B complex content of diet showed decidedly fewer young (4.00) in their litters (feces from animals on low vitamin B diet). Rats impregnated before being placed on a B-free diet were not significantly different from stock in number per litter (7.25 compared with the 8.59 in stock).

TABLE 2

Average weight of young at birth

DIET	NUMBER OF LITTERS	NUMBER OF YOUNG	WEIGHT RANGE	MEAN WEIGHT	PROBABLE ERROR OF MEAN	STANDARD DEVIATION	RANGE / STANDARD DEVIATION	DIFFERENCE OF MEANS / PROBABLE ERROR dif.
			grams	*grams*				
Stock	164	1,379	3.7–6.5	5.27	±0.03	±0.58	5.0	
B-free (when pregnant)	7	51	3.8–5.0	4.34	±0.09	±0.35	3.4	6.6
Feces	7	49	3.0–4.7	3.80	±0.11	±0.53	3.2	8.8

Mean birth weights are significantly lower than stock (5.27) both in litters born on a B-free diet (4.34) (mothers transferred to diet when pregnant) and litters born on a diet with feces (3.80) as a sole source of vitamin B complex (feces from animals on low vitamin B diet).

litter, the average weight of young at birth, and the mortality during lactation on these diets. The mean number of young per litter born on the feces experiment (4.0) is very much lower than on stock (8.59) while rats transferred to a B-free diet (7.25) when pregnant show no significant difference from stock. Young both on the B-free and the feces diets showed very low birth weights, that on feces being the lower (considerable storage

of vitamin B is indicated in the stock mothers transferred to a B-free diet when pregnant). Mortality of young during lactation was 100 per cent for both experimental groups, except for three young transferred from the B-free to stock mothers during the third week (these developed normally). Most of the deaths occurred during the first week. On the feces diet all died within two days with the exception of five young from one litter which survived to the third and fourth weeks and then died.

TABLE 3

Mortality of young during lactation

DIETS	NUMBER OF LITTERS	NUMBER OF YOUNG	PER CENT OF TOTAL DEATHS DURING LACTATION								TOTAL MORTALITY	
			First week		Second week		Third week		Fourth week			
			Mortality									
			Number	Per cent	Number	Per cent	Number	Per cent	Number	Per cent	Number	Per cent
Stock	189	1,624	66	82.5	4	5.0	7	8.8	3	3.8	80	4.98
B-free (when pregnant)	8	58	45	77.6	7	12.1	1 (3*)	6.9	2	3.4	55 (3*)	100.00
Feces	11	46	41	88.0			2	4.3	3	6.5	46	100.00

* Three young from the B-free diet were transferred to stock for cures during the third week. These were in bad condition and would have died had they been left with their mothers. They are therefore counted as mortalities in this table.

This table demonstrates that 4.98 per cent of 1624 "stock" young died during lactation whereas 100 per cent of the other two groups died. Also 77.6 per cent or more of the mortality before weaning occurred during the first week.

SUMMARY

1. The feces from ten rats on a diet supplying 1 per cent yeast was found sufficient to keep the majority of 25 young rats alive for over eight months when used as their sole source of vitamin B.

2. Eleven litters, totaling 46 young, were obtained over a period of several months from parents raised on a diet which supplied feces as a sole source of vitamin B.

3. Statistical results of data on litters from *a*, stock; *b*, the feces diet, and *c*, a B-free diet (females transferred from stock when pregnant) are given. The litters on the feces diet had decidedly fewer young and a very low birth weight. On the B-free diet the number of young per litter was not significantly different from that in stock, but the birth weights were much lower.

4. The mortality of the young during the lactation period was 100 per cent in both the "feces" and the B-free groups.

5. In all groups over 77 per cent of the mortality before weaning occurred during the first week.

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STUDIES IN THE B VITAMINS

V. A Study of Myelin Degeneration in the Peripheral Nerves of Rats as Associated with Low Vitamin B Content of Diet

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A very extensive literature exists on the subject of pathological changes in the nervous system of animals suffering from vitamin B deficiency. No attempt is made here to go into this literature in detail. The studies of Jackson (1) and of Stern and Findlay (2) give historical reviews of the most important work done up to 1929. These indicate the diversity of findings and opinions among different workers in this field. Myelin degeneration in the peripheral nerves of young rats on low vitamin B diets has been reported by this laboratory (Moore, Brodie, and Hope, 3).

Since our work for the past several years has been concerned with the study of various phases of vitamin B deficiency, using relatively large numbers of animals, the time was considered propitious to make an extended investigation of myelin degeneration of the peripheral nerves of rats as associated with low vitamin B content of the diet. The selection of typical animals from our various diet groups enabled us to obtain a generalized and carefully controlled picture.

The study here presented was made on a total of 1,714 slide preparations obtained from 471 rats chosen from 19 of our diet groups (4).

We hoped from this series to determine whether myelin degeneration occurred with sufficient regularity—if at all—to be considered as a valid criterion of vitamin B deficiency in rats.

In the previous work of our own laboratory the findings had been varied, at times no changes being noted, and at others what appeared to be Wallerian degeneration being found. We hoped to discover, if possible, the cause of this discrepancy. We wished to determine whether true Wallerian degeneration could be found in animals of any age on any of our diets. The possibility of apparent myelin degeneration occurring due to such factors as post-mortem changes or mechanical injury occasioned by methods of handling the nerves was investigated. (The latter was suggested by McCarrison in his criticism of the degeneration test (5).) Also the rate

and extent of myelination of the nerves in young animals on stock and experimental diets was compared.

It was recognized that a more rapid post-mortem degeneration or a retarded myelination in the nerves of rats on poor vitamin B diets might be almost as significant as a true degeneration.

Animals used: Typical animals of varying ages were taken from stock and from 18 experimental diets. For the sake of brevity in reporting these, individual diets are not enumerated, but those which were similar have been grouped under appropriate general headings. Four hundred and seventy-one rats were employed of which 83 died or were killed in extreme stages of paralysis or inanition. A total of fifty young taken from the different diets were used in the myelination study.

Killing of animals: Part of the rats used had died from accidental mechanical injury. A few were killed by cutting the jugular vein or by etherizing lightly and then cutting the jugular vein. Most of our animals, however, were etherized. In some of these the nerves were removed while the animals were still living, and in some after death. Other animals died of inanition or paralysis produced by diets deficient or lacking in B_1, B_2, or the B complex. No variations could be demonstrated in the nerve preparations as a result of these different methods of killing the animals.

Nerves: In all cases, except those indicated, nerves were taken immediately or very soon after death. Only peripheral nerves were used, the most common being the sciatics, the phrenics, the vagi, and the brachial plexuses. The larger branches were ordinarily studied, no investigation being made of the nerve endings. (For a consideration of nerve endings in rats on a low vitamin B diet see Woollard, 6.)

Technique of nerve preparations: After some preliminary work with the Marchi and the sodium iodate techniques, osmic acid stain for peripheral nerves was employed. Three of the common variations of fixation were used: 1, fixation and staining with osmic acid solution; 2, short fixation (6 to 12 hours) in formalin with subsequent staining with osmic acid solution; 3, long fixation in formalin (over 12 hours), staining with osmic acid solution. All nerves were preserved in glycerin and teased and mounted in glycerin jelly. Teased nerves were used exclusively in this study; no sections were made.

The Marchi method, although eminently satisfactory, proved impractical for the extent of the work with the limited facilities at our command. The sodium iodate method we found not to be dependable in our hands. The osmic acid stains were simple and reliable, the osmic fixation being somewhat preferable as it was free from the artefacts of the formalin fixation.

Slides and examination: An average of four slide preparations was made from each animal, although there was considerable variation; 1,714 slide preparations were made. These were examined individually by two

workers and separate reports made before notes were compared. The results agreed on all essential points.

Control Wallerian degeneration: Wallerian degeneration controls were prepared using three young adult stock animals. Each rat was lightly etherized, a small incision made in the left leg, and the sciatic nerve trunk cut. At five and ten days the animals were again etherized and portions of the nerve peripheral to the point of sectioning were removed. On the tenth day portions from the whole right sciatics were also taken for comparison (these were normal).

Nerve preparations made from the degenerated nerves were used as a standard of true Wallerian degeneration.

Diet groups investigated: Typical animals chosen from the various diet groups are best listed in the following table.

DIET GROUP	AGE (MONTH)			TOTAL ANIMALS	RATS SHOWING	
	1 to 4	4 to 12	Over 12		Paralysis	Inanition
Stock	57	52	29	138		
10 per cent yeast	25	36	3	64		
2 per cent yeast	12	15		27		
B_1+B_2 diets	58	31		89	18	8
B_1 diets	24	3		27	2	8
B_2 diets	17	12		29	12	2
B-free	21	26		47	4	17

(Fifty young, of which 12 were paralyzed, used in the myelination studies are not included in this table.)

In none of the nerve preparations made from these animals was any condition resembling Wallerian degeneration apparent. We did find in some cases, however, a foamy appearance similar to that described by Schaumann (7) in nerves of rats on a generally low vitamin diet. Also some swelling of the myelin sheaths was found, as in the work of Hofmeister on rats suffering from vitamin B deficiency (8). However, both of these conditions appeared as frequently in our preparations from animals on stock and adequate vitamin B diets as in those deficient in vitamin B. We cannot therefore postulate a causal relationship between these myelin changes and an inadequate supply of vitamin B in the diet. No consistent changes were found to characterize nerves of animals on low vitamin B diets.

Study of post-mortem changes in nerves: Sixteen adult rats, 10 normal from stock, 3 normal from our 10 per cent yeast diet, and 3 suffering from advanced malnutrition on a B-free diet were used. The animals were killed with ether. Small control sections were taken from one set of nerves immediately and the bodies were kept at room temperature. At

the intervals indicated below, later sections were taken from cut nerves (both distal and proximal ends) and from the uncut nerves. Sciatics, brachial plexuses, vagi, and phrenic were used.

A list of the slides prepared follows.

Hours	Immediate	2	5	6	8	12	18	24	30	36
Slides	16	73	90	71	36	16	16	8	8	10

Results: No clear degeneration was discernible until twelve hours or more had elapsed. From that time it became more and more evident, so that by twenty-four hours it was very marked. No difference could be demonstrated between cut or uncut nerves. Nor was there variation in the time of appearance or extent of degeneration for the different diet groups.

Mechanical injury of nerves: For this study adult animals were taken from all of the synthetic diets and a complete age range (after weaning) of stock rats. In each case as long a portion of the nerve as possible was carefully removed from the animal. One or two sections were always saved as controls, one portion was dried until quite stiff and brittle, one crushed with forceps, and one stretched vigorously. When teasing the nerves, adjacent portions of one or more control segments were reserved for heating. In approximately half of the cases the nerves were examined in glycerin jelly before heating, then heated somewhat and again examined, and the process repeated. This produced a perfect control, making it possible to see changes in individual fibers.

A list of the preparations made follows.

	CONTROL	DRIED	STRETCHED	CRUSHED	HEATED
Stock	80	14	42	41	7
Experimental	123	25	101	67	17

Results: The nerves heated slightly showed no variation from the controls. If heating was continued they shrank and became twisted. Due to shrinkage they became darker. If heating was extensive they twisted into very compact bunches and became black or almost so. As long as the nerve structure could be determined it was like the control. In no case could a normal nerve after heating have been mistaken for a degenerated one.

Nerves if dried slightly showed no consistent variation from the controls.

With extensive drying they became very compact and were teased with difficulty. The stain was dark or black, the nerves brittle, breaking as readily across as between the fibers. The microscopic appearance of these nerves in no way resembled myelin degeneration.

Crushing the nerves tended to break the myelin and in some cases caused extrusions of myelin through the sheath. This was widespread or hardly noticeable depending on the severity of treatment. However, the myelin damage caused by crushing was sharp and irregular, showing none of the characteristic globular particles found in degeneration. The ordinary observer would not mistake one for the other.

After stretching, if not held taut during fixation, the nerves twisted extensively. Pulling tended to break the nerves or the myelin within the nerves, but there was no appearance simulating degeneration due to it.

It was possible to differentiate grossly between the unteased nerves by their appearance and feeling. Dried nerves were stiff, relatively thin, and rather brittle. Crushed and stretched nerves were soft, pliable, relatively large, and broke into their fibers readily. The controls (untreated nerves) ranged between the two.

These differences persisted for at least fourteen months when the nerves were preserved in glycerin after staining.

No variation in appearance could be demonstrated between nerves from the different diet groups.

We conclude that mechanical injury due to heating, crushing, stretching or drying of the peripheral nerves does not produce changes in them which could be interpreted as myelin degeneration.

Myelination studies: Our interest in this subject depended on its influence on qualitative and practical histological tests as concerned: *a.* The possible mistaking of myelination for myelin degeneration. *b.* The possible retardation of myelination in rats on diets deficient in vitamin B. Should the second possibility prove apparent a thorough quantitative study would, of course, be indicated in view of its interesting theoretical possibilities. Our investigation of myelination of the peripheral nerves of the rat was in no sense undertaken as a re-checking of the quantitative work done in this field at the end of the last century (8). (Our technique could show only qualitative differences.)

The following preparations were made of the nerves of young from stock litters and young killed and dying from litters whose mothers were on low vitamin B diets. Fifty young were used, of which, on the low B diets, 12 showed typical paralysis. The young obtained at two weeks gestation were taken alive by Caesarean section on normal stock mothers.

Nerve preparations studied

	GESTATION TWO WEEKS	BIRTH	WEEKS TO WEANING			
			One	Two	Three	Four
Stock	8	8	11	42	59	22
Low B diets			8	12	40	22

In premature young taken by Caesarean section at two weeks' gestation or in young at birth no myelination in the peripheral nerves could be demonstrated. As had been shown by earlier workers (8), myelination was found to be slight at one week, to advance steadily during the next two, and to be practically completed by the fourth. The forming myelin globules as seen in the teased sections obviously resembled myelin degeneration and could readily be mistaken for that process. It seems probable that without careful controls the myelination process in young rats may be misinterpreted as myelin degeneration.

The nerve preparations from paralyzed young were not distinguishable from the controls. By our methods no indications of retarded myelination associated with a deficiency of vitamin B could be shown even in the case of paralyzed young.

SUMMARY AND CONCLUSIONS

1. This study was made on 471 rats, chosen as typical, from our stock and 18 of our synthetic vitamin B diets. Of these, 83 animals died or were killed in late stages of inanition or paralysis due to vitamin B deficiency. Only peripheral nerves were studied.

2. A total of 1,714 slide preparations was made, using the osmic acid staining and teased nerve techniques exclusively.

3. No true Wallerian degeneration was seen in any of the nerve preparations other than in the Wallerian degeneration controls and late post-mortem preparations.

4. A foamy appearance of the myelin and swelling of the myelin sheath was seen as frequently in our controls as in our low vitamin B diets. No causal relationship between these changes and an inadequate supply of vitamin B in the diet could be demonstrated.

5. A study of post-mortem changes in nerves left in the body at room temperature showed that no myelin degeneration appeared until 12 hours or more had elapsed.

6. No acceleration of post-mortem degeneration was demonstrated in nerves of rats on deficient vitamin B diets.

7. Mechanical injury of nerves occasioned by heating, drying, crushing, or stretching did not produce myelin changes simulating Wallerian degeneration.

8. The myelination process might readily be misinterpreted as myelin degeneration in young rats.

9. Myelination was not found to be retarded in young rats on low vitamin B diets, by the methods used.

10. No consistent changes in the nerves were found by our methods to characterize vitamin B deficiency in rats.

11. We do not consider myelin degeneration in the peripheral nerves of rats a valid criterion of vitamin B deficiency.

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STUDIES IN THE B VITAMINS

VI. Further Consideration of Pyloric Obstruction in Rats

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For several years the Nutritional Research Laboratory of the University of Oregon Medical School has been studying problems connected with the effect on the young of inadequate maternal diets during pregnancy and lactation, especially the effect of a deficiency of the vitamin B complex or of B_1 or B_2. From 1925 to 1927 the effects on litters of 2 per cent yeast as the source of vitamin B in pregnant and nursing rats was investigated. This diet was found to be insufficient for normal production and growth of litters in our animals, being associated with hemorrhages of the young and the mother, lowered birth weights, lower weight gains, high mortality, and some polyneuritic paralysis, especially in second generations on this diet (1).

These findings, although indicating a greater need for the B complex in our series of animals than some other laboratories had reported (2), were in line with recognized results of a vitamin B deficiency and were in part corroborated by other workers (3). However, we were surprised to find the appearance of a condition simulating pyloric stenosis in ten of our young on this diet (1.2 per cent of the young in the first generation, 22 per cent of the young in the second generation, 87.5 per cent of all cases being males). This pyloric obstruction was found in none of our normal diets nor in any of our higher percentage yeast diets carried on at the same time. Treatment and subsequent history of the rats developing this condition indicated that there might be a definite relation between it and the food deficiency.

As the personnel of the laboratory was to be changed at the time, it was deemed advisable to report the findings, although the investigation was clearly in its preliminary stages.

In the first paper (4), an experimental study by Brodie, the pyloric obstruction found associated with low vitamin B intake in our rats was discussed in detail. In the second (5), a clinical study by Moore, Brodie, Dennis and Hope, the history and literature of congenital pyloric obstruc-

tion was reviewed. In both papers the possibility of interpretation of the condition as due to a dietary lack of vitamin B, possibly associated with an hereditary tendency, was pointed out. It was obvious that the results were too incomplete to warrant the drawing of definite conclusions; but the indications were clearly worth calling to the attention of other investigators in the field, particularly to clinical workers who had available cases which they could examine and treat with a view to verifying or refuting this vitamin B deficiency hypothesis.

Congenital hypertrophic pyloric stenosis has long been known to be common in particular families, some workers believing it to be actually hereditary (6). In our papers presenting the hypothesis of vitamin B deficiency as the cause of pyloric stenosis it was pointed out that this heredity might be apparent or real, i.e., due to family food habits or to a distinct hereditary need of a high B intake (5). Pathology in vitamin B deficiencies has been found to be so varied and extensive that eventually it may be shown that too low an intake tends to precipitate any of several conditions where there is a predisposition of certain parts of the body to weakness.

Soon after the work above had been done the personnel of the laboratory was changed, and numerous alterations of methods and policy were introduced. Primary among these was the method of administering the vitamin portion of the diet. Previously, a 2 per cent yeast diet had consisted of 2 per cent of the yeast being mixed grossly with the basal food. In this way each animal received a quantity of yeast directly proportional to the amount of basal food he consumed, thus causing a variation from day to day for an individual animal, and between different animals of the group. In order to limit definitely the amount of vitamin B being utilized, a preliminary study to ascertain the approximate average amount of food consumed by each animal was carried out. On this basis it was determined that ten grams of food per animal per day was a generous allowance, and our vitamin portions were calculated on this amount. Thus, an animal receiving 2 per cent yeast as a sole source of vitamin B was now given an accurately measured amount of a yeast suspension in tap water equal to 0.2 gram of yeast daily. Small dishes were obtained, and the entire daily vitamin portion (including cod liver oil (A and D), wheat germ oil (E), and the various measured amounts of yeast (B complex) or of our B_1 or B_2 concentrates depending upon the diet) was accurately measured and served to each animal before the basal portion was placed in the cage (7). In this way we were able to control accurately the vitamin intake of each animal independently of the amount of basal food consumed. There was no attempt made to control the latter factor in any of our experiments, adequate amounts being kept in the cages at all times.

Following the introduction of this method of feeding, three important changes in the clinical results obtained in the animals on our 2 per cent

yeast diet (now 0.2 gm. daily per animal) were noted: 1, almost total disappearance of excessive hemorrhage in the newborn and in the mother at parturition; 2, disappearance of cases simulating pyloric stenosis; and 3, decided reduction in the percentage of cases of typical spastic paralysis, with more of the animals dying of inanition with little or no hypertonicity. These changes we are inclined to ascribe to the fact that the animals now received consistently accurately measured amounts of vitamin B complex and that this amount in all probability was in excess of that consumed by the animals in the earlier experiments.

One of the new experiments instituted at this time was carried out in an attempt to analyze the separate rôles played by the B_1 (F or antineuritic) factor and that of the B_2 (growth G or P-P) factor of the vitamin B complex. The facilities of the laboratory did not permit nor did the nature of our work require absolute purity of our preparations, as we were primarily interested in securing such products as would permit of maintenance of our animals to maturity and if possible pregnancy and lactation of the young to weaning.

Considerable preliminary standardization of the methods of securing and the testing of the potency of our preparations resulted in the adoption of the methods described in paper III of this series (8). In this paper it was pointed out that our B_1 preparation was apparently relatively pure, but that our B_2 preparation (autoclaved yeast) probably contained small amounts of B_1 and also of some other factor or factors then unknown.

On one of our diets, consisting of 1 gram per animal per day of autoclaved yeast (B_2) without the addition of any B_1 concentrate or any other source of vitamin B, one litter was obtained. The mother had been on the following diet for almost nine months previous to impregnation: McCollum's basal mixture 95 per cent, McCollum's salt mixture 4 per cent, cod liver oil, and 1 gram autoclaved yeast daily (no vitamin E was supplied).

This female had grown normally and appeared healthy. Her hymen did not open until she was approximately six months old and her oestrous cycles were very irregular. After eight months she was impregnated (by a stock male) and had a normal pregnancy. A detailed protocol on her delivery is given below.

Protocol of delivery of female 855, mated to male 1030, September 1, 1928; delivery September 24, 10:15 a.m. (dictated at the time the young were being cared for).

One male young was delivered 10:15 a.m., out of the nest, covered with sawdust, and apparently moribund, gasping for breath every eight to ten seconds without normal breathing reflexes. It was washed with warm water and artificial respiration was administered with the thumb and forefinger at the rate of about seventy per minute for approximately fifteen minutes. Gasping increased progressively until labored inspiratory movements of the diaphragm were begun at about ten minutes; these became progressively more regular until breathing was fairly normal at the end of twenty minutes. The animal was pale and edematous, but this gave way to a normal pink appearance after breathing began.

The cage was again examined (about 10:35 a.m.). Four more young were discovered, breathing normally but covered with sawdust and very cold. These were washed in warm water and warmed in the hand for a few minutes after which they appeared normal.

The cage was again examined, and one female young was found covered with mucus and sawdust, apparently dead. The mother seemed in the act of devouring it, and to prevent her doing so her jaws had to be opened forcibly. The young rat was washed in warm water, mucus cleared from nose and mouth, and artificial respiration administered. Twelve minutes sufficed to elicit the first diaphragmatic efforts, and in fifteen minutes the animal was breathing alone with movements slow and labored but regular.

One more young was found covered with sawdust but breathing normally. No other young were palpable in the mother at 11:25 a.m. The litter contained 4 males, 3 females, no runts, all apparently normal after this treatment. Weight of 7 young, 36 grams. (Average, 5.14 gm., compared with 5.27 gm., stock animals, falls in lower normal range.)

Average litter weight gains during lactation were: one week, 5 grams; two weeks, 12.3 grams; three weeks (5 rats), 13.5 grams; four weeks (1 rat), 17.0 grams. When compared with stock weight gain averages (one week, 6.20; two weeks, 13.63; three weeks, 22.16; four weeks, 31.72) the first two weeks fall in the lower normal range, the last two are definitely subnormal. The mother gained slightly but steadily throughout lactation, showing no ill effects.

The young appeared in excellent condition until the nineteenth day, when it was noticed that they were less lively than before. However, no clinical symptoms of polyneuritis were present until the twenty-first day, when two of the males showed paralysis of the hind legs. One of these was killed and preserved as a control. There was no gastric dilatation. It was attempted to feed the other, a male, an aqueous solution of the antineuritic concentrate (B_1). The rat could not take it and subsequently died with all clinical symptoms of polyneuritis. Autopsy showed the stomach to be greatly distended and the intestines empty except for gas bubbles, but no hypertrophy of the pylorus could be demonstrated.

On the twenty-second day three more young, one female and two males, were badly paralyzed and showed rather extensive blood clots about the nose. The mother also showed this condition. The paralysis of the young in this case seemed to affect the forepaws, which were doubled up. (Moving pictures were made at this time and repeatedly thereafter to the time of the complete cure of the one remaining animal.) In attempts to move about, the young rats walked on the dorsum of the paw almost as frequently as on the palmar surface. The hind legs were affected but were not dragged out posteriorly. The young were unable to balance themselves on their feet. A rubbing of the nose indicative of pruritis was very noticeable. The young were in such bad condition by the afternoon that their lives were despaired of. One of them, the female, was killed as a control

(it showed no gastric dilatation) and the other two, males, were treated as described in the following detailed report, taken from notes made at the time of each observation.

First day. At 3:30 p.m. the animals were too weak to take any food or treatment by mouth. They were apparently moribund, lying apathetically on one side and kicking feebly only when touched. The hair of the top of the head of one was clipped off as a distinguishing mark. This animal was treated with an aqueous filtrate of our B_1 concentrate, 0.1 cc. being injected intraperitoneally and 4 drops being forced down the throat. The other animal was treated with a filtrate of a 20 per cent yeast suspension (macerated for one hour), 0.1 cc. being injected intraperitoneally and 4 drops being forced down its throat. Fifteen minutes later like dosages of the respective filtrates were given. The rats made no attempts to move but lay quietly in a cotton nest near the radiator.

Four hours later the procedure was repeated, 0.2 cc. of each filtrate being used intraperitoneally. The rats appeared somewhat improved by this time and licked rather eagerly at the pipette with which they were fed, each of them consuming about 0.5 cc. An attempt was made to feed them a little of the basal diet made very dilute with water, but only a drop or two was taken.

Second day, 9½ hours after initial treatment. At 1:00 a.m. they were again injected with 0.1 cc. of the respective filtrates, given about 1.5 cc. by mouth and about 10 drops of dilute basal food. They appeared still better, although it was very uncertain whether they would live.

At 4 a.m. they were so much improved that the injection was not given. Each took about 0.5 to 0.75 cc. of its filtrate and a considerable quantity of the basal food. They both ate avidly, although the paralysis was still evidenced by their inability to direct the movements of the head—they would lunge awkwardly at the source of food, licking rapidly the while, then jerk back with a semi-palsied movement. When placed on the floor they were able to maintain their balance much better than before and assayed a few steps.

At 7 a.m. the animals were again fed approximately 0.75 cc. of filtrates and a like amount of basal food. They were much improved and attempted to move about with much better success than previously. By 9:30 a.m., their next feeding, they were improving so rapidly that it was noticeable from hour to hour.

At 12:00 noon they were placed with their mother and left there for the afternoon. It was not determined whether they nursed, but they sought the basal food dish and ate eagerly. At 5:30 p.m., in addition to the usual feeding, the rat receiving vitamin B_1 filtrate was also given a small portion of autoclaved yeast (B_2) which was the mother's diet. Feedings were repeated during the night at 7:30 p.m., 10:00 p.m. and 4:00 a.m. Improvement was continuous and the animals were by this time considered out of danger.

Third day. At 8:45 a.m. each rat was placed in a separate cage and given a dish of basal food, 4 drops cod liver oil, the appropriate filtrate, and some autoclaved yeast for the animal receiving the antineuritic vitamin. Both ate well, the one receiving yeast filtrate with great avidity.

At 12:00 noon and at 1:00 p.m. both animals were apparently in excellent condition, although it was noted that the abdomen of the one receiving yeast filtrate was somewhat distended.

At 2:00 p.m. the rats were again examined. The sun by this time had reached the shelf on which the cages had been placed, and both rats were very warm and panting. (Heat stroke?) They were moved to a cooler place, but the one on yeast filtrate lay

on its side and appeared very ill. A few drops of water were given. It licked these but did not recover further. The body was then immersed in a tepid bath, and it was noticed that the abdomen was immoderately distended. The ruffled appearance of the fur had masked the condition previously. A luer syringe was sterilized and a small needle plunged directly through the abdominal and adjacent stomach walls. Through this was aspirated a small quantity of viscous fluid, which proved, on microscopical examination, to be identical with the food mixture given though mixed with mucus. By this time the animal was gasping painfully and seemed to be dying. A small incision through the abdominal and stomach walls was made and the gastric contents drained. This material was thick and mixed with mucus but still fluid. The animal was too severly affected for its life to be saved. Immediate autopsy showed the stomach walls to be thinned out and stretched to an abnormal degree, but the intestines were empty except for gas bubbles and some hard feces in the lower end of the colon and in the rectum. The other animal was in very good condition.

Fourth day. By the next day the remaining animal showed recovery without any apparent complications. It ran about almost normally, had practically cleaned the accumulated food from its coat, and evidenced the usual inquisitiveness of a young rat of this age (twenty-five days). It had not been returned to its mother, but was kept on the food mixture of basal food, autoclaved yeast, cod liver oil, and antineuritic concentrate.

Fifth day. The following day the animal had completely recovered as far as could be determined. It was very lively. Its coat was clean, soft, and fluffy. Except that it was somewhat smaller than normal it showed no differences from our stock rats. At this time it was decided to deprive it again of all B_1, its food now consisting only of basal mixture, cod liver oil, and autoclaved yeast. After three days on a B_1 free diet the rat demonstrated the hypersensitiveness and the gait usual in animals approaching paralysis. Also the abdomen was slightly distended.

By the fourth morning on the B_1 free diet, the little animal was definitely ill. There were hard, sticky feces adherent to the anus. A small amount of warm saline was given as an enema with some slight effect. The abdomen was definitely distended, bulging abnormally on both sides in the sub-costal region, and the rat ran about in evident pain.

By 10:30 a.m. the symptoms had become progressively worse. The rat moved about very quickly, but its movements were not well-controlled. It would not eat or drink. There was embarrassment of respiration. The abdomen was enormously distended. To all appearances the animal would have died within a few hours if not treated.

It was therefore lightly etherized. A small slit was made in the skin over the fundal region of the stomach, the stomach was compressed from the right side, and a small hypodermic needle plunged through the abdominal and stomach walls directly into the fundal portion of the stomach cavity. Attempts to aspirate were futile because of the viscosity of the stomach contents, so a syringe containing antineuritic filtrate was attached and 0.3 cc. injected directly into the stomach. This quantity was not sufficient to have diluted the stomach contents to any appreciable extent. On coming out of the anaesthesia the rat appeared to be no worse than before. At 11:15 a.m. it remained in about the same condition. It had survived the operative treatment very well and showed no ill effects.

By 12:00 noon the abdomen showed a decrease in the distention, and the rat was breathing very much easier. By 2:00 p.m. the distention had subsided to practically normal. The animal did not move about in the same agonized manner, but it ap-

peared weak. When offered the antineuritic filtrate it grasped the pipette tightly with both forepaws and drank eagerly all that it was allowed, 0.5 cc. At 6:00 p.m. another feeding of 0.5 cc. of the antineuritic filtrate and a small amount of very dilute basal food was given. The rat seemed almost normal again.

By the next morning it was apparently entirely normal, and one would never have guessed it to be the same animal treated the day previously. It was again given a dish of dilute basal food, autoclaved yeast, antineuritic concentrate and cod liver oil, of which it ate avidly. Two days after treatment it was lively and well.

One other female in the litter died of paralysis on the twenty-fourth day of lactation. There was no gastric dilatation. The one remaining female of the litter although showing some paralysis at the end of the third week recovered without a change of diet and was weaned.

Although repeated attempts were made over a period of fourteen months, using 41 females, to secure young on this diet, only three litters were obtained. The first of these is reported in detail above. In the case of the other two the deliveries occurred during the night and the young did not live. We were therefore unable to duplicate the findings here reported.

We do not wish to draw any conclusions from this one litter, but we think it worthy of consideration in the light of the earlier finding in this laboratory of cases simulating pyloric obstruction in the young born on a diet containing 2 per cent yeast as a sole source of vitamin B. We feel that in the experiments earlier reported from this laboratory, the females on the 2 per cent yeast diet may have received smaller amounts of yeast than did the later animals which received measured 0.2 gram portions of yeast daily.

It is of interest to note, in comparing this with the other cases of apparent pyloric stenosis reported in this laboratory, that yeast has been shown to be relatively lower in the B_1 than in the B_2 factor (9). The minimal amounts of yeast consumed may then have supplied B_1 in such small quantities as to have influenced the production of the pyloric obstruction. This might also account for the occurrence of gastric distention in the case reported above in an animal cured of paralysis by administration of a whole yeast filtrate.

SUMMARY

1. Earlier papers from this laboratory reported 10 cases (87.5 per cent males) of pyloric obstruction in litters born on our diet containing 2 per cent yeast as the sole source of the vitamin B complex. Other clinical findings on the same diet had been hemorrhages of the young and the mother and a high percentage of deaths with terminal paralysis.

2. A change in methods of vitamin feeding was introduced, 0.2 gram of yeast being fed each animal daily in place of the former 2 per cent yeast mixed with the basal food. Subsequently, excessive hemorrhages were

not found, fewer young died of polyneuritis, and no cases of pyloric obstruction appeared.

3. In a series of experiments extending over a period of 14 months and utilizing 41 females raised on a diet in which 1 gram of autoclaved yeast (B_2) was supplied daily as a sole source of vitamin B, only one viable litter was obtained. This delivery was abnormal.

4. At approximately 3 weeks of age one of the four males in this litter of 7 died showing a condition of gastric dilatation resembling pyloric obstruction accompanying typical polyneuritis.

5. One paralysed male killed as a control showed no gastric dilatation.

6. Complete recovery from spastic paralysis was brought about in one male by early intraperitoneal injections and later oral administration of small quantities of a filtrate of yeast. Approximately 34 hours after the initial treatment, and only a few hours after apparent complete cessation of the paralytic symptoms, this animal rather suddenly developed a condition of gastric dilatation which caused its death in spite of operative attempts to save it.

7. Complete recovery from spastic paralysis was brought about in the remaining male of the litter by similar treatment using a filtrate of the B_1 concentrate. After recovery this rat was again deprived of the B_1 factor for 3 days. On the fourth day immoderate gastric dilatation occurred. Intragastric injection of the antineuritic filtrate caused complete alleviation of this condition within four hours. This animal recovered entirely when continued on B_1 and B_2 in adequate amounts.

8. One female of the litter, although slightly paralyzed, recovered spontaneously and was weaned.

9. No female of the litter showed gastric dilatation.

10. Gastric dilatation was found in 3 of the 4 males.

It would appear that we were dealing with a condition of pylorospasm, probably brought about by a lack of vitamin B_1, as a complete cure was occasioned by treatment with our B_1 preparation.

We present this study in the hope of stimulating the interest of other workers in this line of research.

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THE EFFECT OF COELIAC GANGLIONECTOMY ON THE SUGAR TOLERANCE OF DOGS[1]

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The nervous regulation of carbohydrate metabolism has been reviewed by Pollack (1923) and Macleod (1931). More recent observations following stimulation and section of the vagus and section of the splanchnic nerves have been summarized by us in another article (in press). These contributions indicate that 1, certain types of vagal stimulation produce hypoglycemia, presumably through increased secretion of insulin, and 2, that section of the splanchnic nerves increases the susceptibility of the animal to insulin. In the light of these data it seemed important to examine the effect of coeliac ganglionectomy on sugar tolerance, especially so since previous data were not available in the literature.

METHODS OF EXPERIMENTATION. In a previous article (1930) we have described various trials with oral, intraperitoneal and intravenous administration of dextrose for purposes of determining the sugar tolerance of dogs. By far the most constant data were obtained by the method of Woodyatt, Sansum and Wilder (1915). We have insisted on a possibly complete relaxation of the animal, which was trained to lie quietly in a basal state, during an hour of injection. Excitable dogs, or dogs that were difficult to train to lie quietly for an hour were rejected. "Pfanstiehl" dextrose was used in 5 per cent solution, autoclaved and driven through glass coils immersed in a water bath, so that the solution was introduced at approximately body temperature. The dextrose solution was injected with the help of the Woodyatt pump at timed rates for the period of an hour. A twenty-four hour sample of urine was collected under thymol and the sugar, if present, was determined by Benedict's method. From two to three pre-operative tolerances were determined which checked within a 0.1 gram of dextrose per kilogram body weight. The tolerance was expressed as the maximal amount of dextrose that could be injected during the period of an hour without producing a glycosuria. Post-operative determinations were made at the end of two weeks and subsequently once a

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month. The dogs were observed for at least six months, but some have been followed for over two years.

In some of the earlier experiments blood sugar determinations were also made before, at the end and for several hours after the intravenous injection in order to study the rate of disappearance of the excess of dextrose from the blood stream.

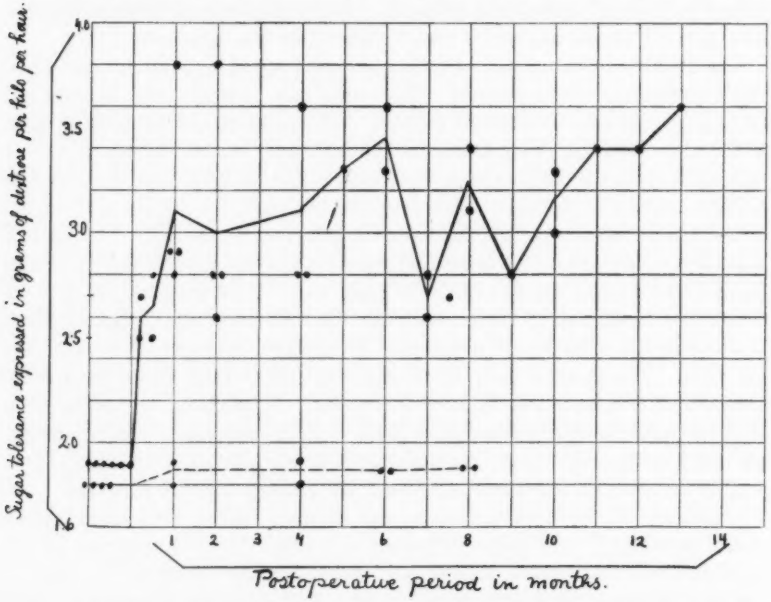
In addition the response of these dogs to insulin was studied before and after operation. Insulin was given in doses of $\frac{1}{10}$ of a unit per kilogram dissolved in 1 cc. of normal salt solution. Blood sugars were determined before, and 10, 20, 30 minutes, 1 and 2 hours after the injection.

The technique of operation has undergone several modifications since the experiments were started. The semilunar ganglia can be reached through an incision which runs parallel to the right or left intercostal arch. When the operation is started from the left side, the suprarenal plexus is picked up just mesial to the left adrenal gland and followed toward the midline, until around the coeliac axis, the left coeliac ganglion and on the other side of the artery, the right coeliac ganglion is reached. Or one can start from the right side, expose and retract the vena cava to the right and remove the coeliac ganglia from the right side. The ganglia can be very definitely recognized by their somewhat different color, which distinguishes them from the subordinate plexuses. Their shape and size, as in man, are variable. The ganglia have to be dissected off bluntly from the coeliac axis, which is comparatively long in the dog. It has been our impression that the most complete excision of these variable structures can be accomplished by exposing them, through one incision, from both sides, thoroughly dividing all connections between the ganglia and the adrenals.

Results. A total of ten dogs was studied before and after coeliac ganglionectomy. The sugar tolerance of normal dogs, determined by the method described above, varied only very slightly between 1.8 and 1.9 gram of dextrose per kilogram per hour. Following the operation, a rise in tolerance occurred in every instance varying, however, between the minimum of 2.8 grams to the maximum of 3.8 grams of dextrose per kilogram body weight. While a rise was present at the first post-operative determination, which occurred at the end of two weeks, there was a tendency to a further rise in tolerance, which seemed to reach its peak at the sixth month. From then on it would fluctuate slightly, influenced by respiratory or skin infections, but generally remained high as long as determinations were made. While the surgical mortality was small, we lost several dogs months later by distemper, so that their data could only be partially utilized. In graph 1, all pre- and post-operative tolerance determinations (40 in number) have been charted. An average curve shows the decided and persistent rise in tolerance following coeliac ganglionectomy. There are considerable variations in the responses of the individual dog.

Two dogs, whose liver had been denervated, do not show a rise in tolerance at any time, in spite of the fact that six post-operative determinations have been made on each animal. These were used as controls.

A study of the blood sugar levels at the end and for four hours after the infusion of 2 grams of dextrose per kilo, shows that in the normal animal, the average blood sugar rose to slightly over 300 mgm. per 100 cc. at the end of the dextrose infusion. An hour later, the blood sugar was normal. Following coeliac ganglionectomy, the peak of the blood sugar level was



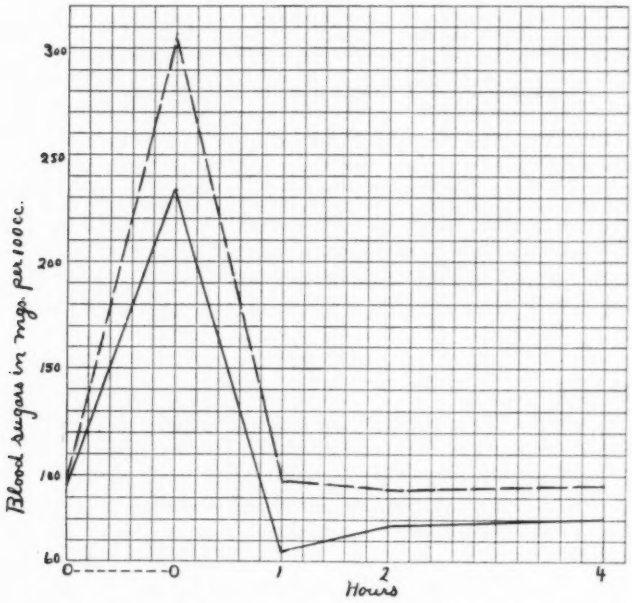
Graph 1. Average curve of the rise in sugar tolerance following coeliac ganglionectomy. An immediate rise takes place following the operation which persists with some fluctuations at the end of a year. The sugar tolerance is expressed in grams of dextrose per kilo per hour. The control curve shows the effect of liver denervation on sugar tolerance.

70 mgm. lower, and a glycosuria did not develop. The blood sugar dropped to a hypoglycemic level at the end of one hour, and returned to normal at the second hour. The rate of disappearance of the injected dextrose from the blood stream is obviously increased after the operation (graph 2).

The response of the operated animals to insulin is definitely increased. The dose of 0.1 unit per kilogram body weight was so selected that it would produce a small dip in the blood sugar level of the normal animal.

Following coeliac ganglionectomy, this dose would produce not only a marked hypoglycemia but quite a delay in the return to the normal level. (table 1).

COMMENT. The method of determining sugar tolerance by an intravenous injection of dextrose at timed rates has given consistent results in our hands. In over fifty determinations on normal dogs, which were made in this series and in other experiments, the rate of utilization proved to be between 1.8 and 1.9 gram of dextrose per kilogram per hour. These figures are much higher than those determined by the originators of the



Graph 2. The effect of coeliac ganglionectomy on the rate of disappearance of injected dextrose from the blood stream.

0--------0: 2 grams of dextrose per kilo were given intravenously for one hour.
Interrupted line: before coeliac ganglionectomy, average of 8 determinations.
Straight line: after coeliac ganglionectomy, average of 5 determinations.

method (Woodyatt, Sansum and Wilder, 1915). We have trained the dogs to permit us a determination in a basal, relaxed state of the animal and we have used "Pfanstiehl" dextrose in a 5 per cent, and in some of the experiments in a 10 per cent solution. All these factors influence the rate of utilization.

Control tolerance determinations, repeated from two weeks to a month, did not raise the tolerance of the dog at any time above 2 grams of dextrose

per kilo per hour. However, following coeliac ganglionectomy, the tolerance rises as early as the first week after the operation, when the dog has not even completely recovered. At the end of a month, the rate of utilization rises to about 3 grams of dextrose per kilo per hour and from then on shows a slight tendency to rise. It has never been observed to drop back

TABLE 1

Showing the increase in sensitivity to insulin which occurs in dogs after removal of the coeliac ganglion

The dogs were given 0.1 unit of insulin per kilogram body weight, dissolved in 1 cc. of normal salt solution, intravenously.

DOG NUMBER	POST-OPERATIVE PERIOD	FASTING BLOOD SUGAR	10 MINUTES	20 MINUTES	30 MINUTES	1 HOUR	2 HOURS
Insulin response following coeliac ganglionectomy							
62	5 days	89	64	46	*41*	60	91
87	2 weeks	80	71	58	*48*	64	80
85	2 weeks	70	51	46	*46*	54	70
86	2 weeks	68	64	46	*40*	45	68
68	5 weeks	67	68	67	53	*46*	67
73	2 months	87	83	54	*44*	62	87
75	2 months	75	74	53	47	*41*	84
77	2 months	70	58	49	46	*44*	69
78	2 months	75	66	52	40	*38*	60
56	4½ months	87	*48*	55	64	89	113
Average values.....		76.8	64.7	52.6	46.9	54.3	78.9
Insulin response of normal dogs							
66		101	90	85	79	87	97
65		98	92	74	68	78	90
64		101	98	72	76	102	95
48		83			76	70	76
50		90			83	75	80
51		87			85	70	73
101		82	86	61	63	72	81
102		71	68	57	57	64	71
103		70	61	53	57	51	71
104		82	65	50	52	68	82
Average.....		93.5	80	64.5	69.6	73.5	81.6

to the pre-operative level, although we have studied one dog for sixteen months.

When the dextrose is injected into the blood stream, the hyperglycemia reaches a lower level after the operation and reacts with a definite hypoglycemia. Whether this means that more insulin is available or that the animal is more sensitive to the same amount of insulin cannot be stated

on the basis of the present data. It appears that the action of extraneous insulin is exaggerated in the ganglionectomized dog. In fact, minute doses, such as 0.1 unit of insulin per kilogram body weight given intravenously, are capable of producing a hypoglycemia of 40 milligrams and less with a visible reaction, following coeliac ganglionectomy. We believe, however, that further experimentation is necessary to establish the value of this method for determining insulin susceptibility.

The mechanism of the action of coeliac ganglionectomy in augmenting sugar "tolerance" needs further analysis. A denervation of the liver, as shown by Donald (1931) and our few observations, does not produce a rise in tolerance. There remains then the action on the pancreas and adrenals. It is more than likely that coeliac ganglionectomy will increase the blood flow to the pancreas, although this problem needs further investigation (1931). It may interrupt vasoconstrictor impulses to the vascular net-work of the islets. In preliminary tests, adrenal denervation alone has also given a marked rise in tolerance, so that coeliac ganglionectomy may operate in that manner. Even though it may not be clear at the present time how removal of the coeliac ganglion leads to an increase in sugar "tolerance" our data yield additional evidence to that already recorded in the literature within recent years, showing that nervous factors (glyco-regulatory impulses) influence insulin production or requirement.

SUMMARY

The effect of coeliac ganglionectomy on the sugar tolerance of normal dogs was studied. The intravenous sugar tolerance of normal dogs does not vary spontaneously under basal conditions. The removal of the coeliac ganglion results in a decided, persistent rise in tolerance in every instance. Intravenously administered dextrose disappears more rapidly from the blood stream than before the operation. The dogs become more susceptible to insulin. Denervation of the liver does not have a similar action. These data show that the coeliac ganglion mediates nerve impulses, the exclusion of which brings about either an increased insulin production or a reduction in the insulin requirement.

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BLOOD URIC ACID FOLLOWING THE INTRAVENOUS ADMINISTRATION OF URIC ACID IN NORMAL DOGS

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The blood and urine of normal dogs contain only minute amounts of uric acid. Following intravenous administration, uric acid disappears from the blood with great rapidity but only a small fraction of the amount given is excreted in the urine. This is apparently true of all breeds of dogs except the Dalmatian. The studies of Mann and Magath (1) and of Bollman, Mann and Magath (2) have shown very conclusively that the liver is the organ primarily responsible for the destruction of uric acid in dogs. Following removal or severe damage of the liver, in dogs, uric acid appears spontaneously in the blood and urine, and parenterally administered uric acid can be quantitatively accounted for in the blood and urine. The exhaustive studies of Folin, Berglund and Derick (3) show that uric acid does not diffuse readily into the general body tissues. Hunter and Givens (4) clearly demonstrated the relationship between the excretion of uric acid and of allantoine. It is now generally believed that in dogs most of the uric acid, of either endogenous or exogenous origin, is oxidized to allantoine and excreted as such.

It follows from the above facts that in dogs the disppearance of intravenously administered uric acid from the blood should be a rather accurate index of the rate at which uric acid is destroyed or oxidized to allantoine.

The results reported in this paper are the first of a series of experiments planned to study uric acid metabolism in dogs and human subjects by means of the blood uric acid tolerance curves following intravenous administration of uric acid.

METHODS AND PROCEDURE. All of the experiments reported in this paper were performed upon the same dog—a mongrel male weighing approximately 12.2 kilos. During the period of about eight months occupied by the experiments the animal was maintained upon a low purine diet consisting of fresh whole milk and karo syrup in quantities sufficient to cover the energy requirements. The animal was routinely fed at 4:30 p.m. each day. On days preceding experiments no food was given but water was allowed ad libidum. During the entire experimental period the

animal was kept in a small individual metabolism cage in warm animal quarters. During the early part of the work the animal developed distemper but after a period of approximately two weeks made an uneventful recovery. With this exception the animal remained in good health and showed a definite gain in weight during the experimental period.

The urine collections were carried out as follows: About 2:00 p.m. on the day preceding the experiments, the bladder was emptied by catheter and the animal placed in a metabolism cage. The following morning, just before the intravenous injection of uric acid, the bladder was emptied by catheter and the urine added to the amount already collected. Following the injection of uric acid, the urine was collected by catheter every two or three hours for the next six or eight hours, after which the animal was placed in a metabolism cage and the urine collected for the remaining part of the twenty-four hour period at the end of which the bladder was again emptied by catheter.

Blood uric acid determinations were made by the new method of Folin (6) using unlaked blood (5). As pointed out by Folin, this method is very accurate and is free from practically all of the errors inherent in the older methods.

The uric acid was administered intravenously in a solution originally recommended by Koehler (7). This solution contains 1.0 gram of uric acid, 0.28 gram of lithium carbonate and 1.35 gram of glucose per 100 cc. By increasing the lithium carbonate in proportion to the uric acid, we were able to obtain a solution containing as high as 1.2 gram of uric acid per 100 cc.

When single intravenous injections of uric acid were given the required amount of the solution was injected into the jugular vein. The time occupied by the injection was usually from one to three minutes. Blood samples were then collected at stated intervals from the opposite jugular vein.

When continuous uric acid injections were employed the solution was injected into the saphenous vein by means of a simple gravity controlled injection apparatus which permitted the flow per minute to be accurately regulated. Blood samples were collected from the jugular vein.

Results. All experiments performed may be divided into three groups as follows:

I. *Single injections.* In these experiments the uric acid was given in a single injection. The total quantities of uric acid injected in the various experiments were 0.640, 0.860, and 1.20 gram. The time required for the injection varied from one to three minutes and following the injection blood samples were drawn as rapidly as possible for varying periods of time.

II. *Continuous injections.* In these experiments the uric acid solution was injected into the saphenous vein as described above. The total

quantities of uric acid injected in the various experiments were 1.20 gram; 1.65 gram and 1.80 gram. The rate of injection was regulated so that the amount of uric acid administered was 10 mgm. per minute. This rate of injection was maintained for periods of two to three hours in the various experiments. Blood samples for analysis were obtained from the jugular vein during the continuous injection.

III. *Combined single and continuous injections.* In these experiments the continuous injection was given into the saphenous vein as described above. The rate of the continuous administration was 10 milligrams of uric acid per minute. The continuous injection was maintained throughout the duration of the experiment. About 45 to 60 minutes after the start of the continuous injection, a single injection was given into the jugular vein in the usual manner. The amount of uric acid given in the single injection was usually 0.8 gram. This amount was injected in from one to two minutes.

Each of the above types of experiments was repeated many times and the outstanding feature of the results was the remarkable agreement between the individual experiments in each type. In reporting the results one curve illustrating each type of experiment will be given and any important variations in other experiments will be mentioned.

(A) *Single injections.* A total of ten experiments was performed in which the uric acid was given in a single injection. Figure I shows the blood uric acid curve following a single injection of 0.640 gram of uric acid. In analyzing this curve it was found that when the logarithm of the milligrams of uric acid per 100 cc. was plotted against the time after injection the result was a straight line (fig. II). The rate of decrease is about 3 per cent of the amount present per minute. Figure III shows the blood uric acid curve following a single injection of 1.20 gram of uric acid. In this experiment the blood uric acid was determined until it reached 0.7 mgm. per 100 cc. In order to determine such small quantities, the usual quantity of the protein-free filtrate was made up to a final volume of 12.5 cc. instead of the usual 25 cc. Figure IV shows the result of replotting this curve with the logarithms of the concentrations of the blood uric acid as the ordinates and the time after injection as abscissae. It is seen that the blood uric acid decreases in a straight line for 57 minutes after the end of the injection, the rate of decrease being 4.5 per cent of the amount present per minute; after 57 minutes, or when the concentration of blood uric acid becomes less than 1.5 mgm. per 100 cc., there is a break in the curve and the rate of disappearance of uric acid from the blood drops to about 1.7 per cent of the amount present per minute. This break in logarithmic curves when the concentration of the reacting substances becomes very low is a common occurrence. We have no experimental proof as to its cause in these experiments but at least three possible ex-

planations may be mentioned: first, it may signify that there are two processes concerned in the disappearance of the uric acid from the blood one of which is much more rapid than the other, in this case, the first part of the curve would represent the sum of both processes with the more rapid process predominating while the second part of the curve would represent the slower process; second, in vitro experiments on enzyme actions usually show a decrease in the rate of transformation when there is an accumulation of the end products of the particular enzyme reaction; while it is not impossible for this to occur in the intact animal, it nevertheless appears quite unlikely; third, with very low concentrations the rate of disappearance may be secondarily governed by the rate of blood flow through the organs concerned with the disappearance of uric acid from the blood, this factor being relatively unimportant when the concentration of uric acid in the blood is high. Our data do not enable us to distinguish between these possibilities.

All of the single injection experiments performed gave results similar to those shown in figures I to IV. Following the injection the blood uric acid decreased logarithmically; when the concentration became as low as 1.5 to 2.0 mgm. per 100 cc. there was a decrease in the rate of disappearance. The rate at which the uric acid disappeared from the blood, before the break in the curves, varied between 6.5 per cent and 3 per cent of the amount present per minute; after the break the rates of disappearance varied between 3.3 and 0.9 per cent per minute. There was relatively little difference in the rates of disappearance from the blood when the quantity of uric acid injected varied from 0.640 to 1.20 gram. In one experiment performed while the animal was acutely ill with distemper in which 0.640 gram was injected, the rate of disappearance from the blood was only 1 per cent of the amount present per minute. This was the slowest rate of decrease in blood uric acid noted in any of the experiments. An experiment performed about one month later, after complete recovery from distemper, and in which the same quantity was injected showed a decrease of 3 per cent per minute which was well within the normal range. The effect of fasting for 5 days and of feeding a meal of bread, karo syrup and milk one-half hour before the experiment were tried but neither of these procedures caused significant alteration in the results.

The per cent of the uric acid which was excreted in the urine in these experiments was very small and varied between a maximum of 5.5 per cent in an experiment in which 0.640 gram was injected, and a minimum of 2.7 per cent with injection of the same quantity. The average for all experiments was 4.1 per cent of the amount injected. The actual quantities excreted varied from 0.023 gram to 0.044 gram when the amount injected was 0.640 and 1.20 gram respectively. From these figures it is evident that the amount excreted in the urine was negligible. From 95

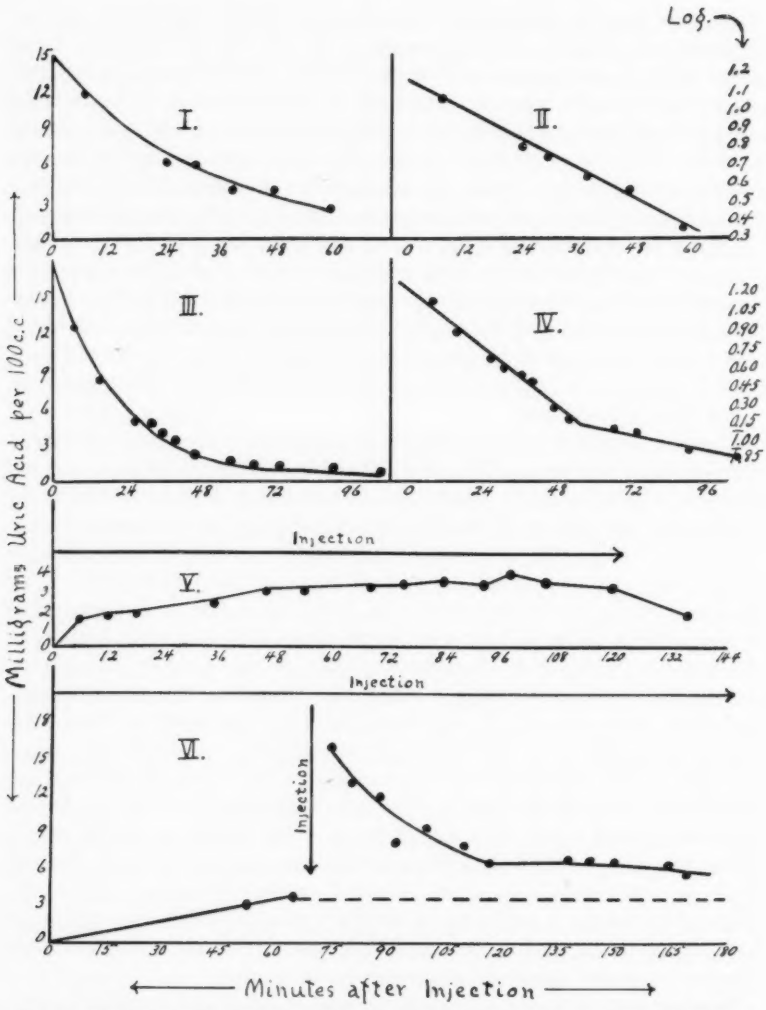

Fig. I. The blood uric acid following a single intravenous injection of 0.640 gram of uric acid. Five and five-tenths per cent of the amount injected was excreted in the urine.

Fig. II. The curve shown in figure I replotted with the logarithm of the blood uric acid concentration against the time after injection. The rate of disappearance is approximately 3 per cent per minute.

Fig. III. The blood uric acid following a single intravenous injection of 1.20 gram of uric acid. Three and seven-tenths per cent of the amount given was excreted in the urine.

to 100 per cent of the total quantity excreted in the urine was secreted during the first three or four hours after injection. In two experiments the urine was collected, by catheter, every two hours for twenty-four hours after the injection in order to be certain that there was not a second period of increased excretion, but it did not occur in either experiment.

The results of these single injection experiments show quite clearly that when quantities of uric acid varying from 0.640 to 1.20 gram were administered to normal dogs, very little was excreted in the urine and that the blood uric acid follows a logarithmic curve, the rate of decrease being approximately 3 per cent of the amount present per minute.

(B) *Continuous injection.* In these experiments the solution injected usually contained 10 mgm. of uric acid per cubic centimeter and was injected into the saphenous vein at a rate of approximately 1 cc. per minute. Blood samples for analysis were withdrawn from the jugular vein. Figure V shows a typical experiment in which the injection was continued for 123 minutes. Other experiments were continued for as long as three hours. The results were strikingly similar in all experiments. The blood uric acid began to rise almost immediately after the start of the injection and usually reached its maximum height in from 45 to 60 minutes after the start of the injection, from this time on to the end of the injection a steady state was maintained in which the blood uric acid was very constant. In five such experiments the value of the blood uric acid during the steady state averaged from 2.8 to 3.3 mgm. per 100 cc. and this level was maintained as long as the injection was continued. As soon as the injection was discontinued the blood uric acid began to fall but the decrease was usually very slow. In one experiment in which the injection was maintained for 183 minutes, the blood uric acid dropped from a steady state of 3.3 mgm. per 100 cc. to 1 mgm. per 100 cc. during a period of 63 minutes after discontinuing the injection.

The quantity of uric acid excreted in the urine during the continuous injection was definitely greater than in the single injection experiments and varied between 8 and 9 per cent of the quantity injected or in actual

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Fig. IV. The curve shown in figure III replotted with the logarithm of the blood uric acid concentration against the time after injection. From 0 to 57 minutes rate of disappearance is approximately 4.5 per cent per minute; from 57 to 106 minutes the rate of disappearance is approximately 1.7 per cent per minute.

Fig. V. Continuous injection of uric acid at the rate of 10 mgm. per minute. The steady state reached after 46 minutes averages 3.3 mgm. per 100 cc. A total of 1.20 gram was injected and 8 per cent was excreted in the urine.

Fig. VI. A continuous injection at the rate of 10 mgm. per minute maintained for 183 minutes. A single injection of 0.8 gram given 72 minutes after the start of the continuous injection. Dotted line shows the probable level of the blood uric acid as maintained by the continuous injection. Approximately 36 per cent of the 0.8 gram given in the single injection was excreted in the urine.

amounts from 0.096 to 0.150 gram. The greatest excretion usually occurred during the period of injection and only small quantities were excreted after the injection was discontinued.

The very slow return of the blood uric acid to the zero level following the cessation of the injection and the greater amount excreted in the urine both suggest that the paths of uric acid destruction are possibly clogged by a continuous injection over long periods of time. In some but not all of the continuous injection experiments the animal became nauseated and vomited during the injection. The vomitus was found to contain uric acid.

(C) *Combined single and continuous injections.* Since it was possible to maintain the blood uric acid at a constant level of approximately 3.2 mgm. per 100 cc., by means of a continuous injection, it was decided to study the result of a combined continuous and single injection. An example of this type of experiment is shown in figure VI. The continuous injection was usually maintained for about one hour before the single injection was given, at which time the blood uric acid had usually reached the steady state at a level varying between 3 and 4 mgm. per 100 cc. At this time the single injection of 0.8 gram of uric acid was given into the jugular vein. The continuous injection was maintained during the entire experimental period. In figure VI the probable level of the steady state has been represented by the dotted line at 3.3 mgm. of uric acid per 100 cc. Following the single injection of 0.8 gram of uric acid the blood uric acid rose to 15.7 mgm. per 100 cc. and then began to fall rapidly, the general exponential nature of the curve being similar to the curves shown in figures I and III. The rate of the decline for 45 minutes after the single injection was approximately 2 per cent of the amount present per minute, which is only slightly slower than the rate in the ordinary single injection experiments. It is important to note, however, that following the single injection the blood uric acid did not return to the predicted basal level, since at 98 minutes after the single injection the blood uric acid was still 2.5 mgm. above the theoretical basal level of 3.3 mgm. per 100 cc. maintained by the continuous injection. This delayed return is emphasized when this experiment is compared with the experiment shown in figure III in which the blood uric acid had decreased to less than 1 mgm. 98 minutes after a single injection of 1.20 gram of uric acid.

It was rather difficult to calculate exactly how much of the 0.8 gram given in the single injection was excreted in the urine. However, by averaging the per cent excreted in several continuous injection experiments and subtracting this amount from the total excreted in the combined continuous and single injection experiments we were able to calculate that from 20 to 36 per cent of the 0.8 gram given in the single injection was excreted in the urine. This is very greatly in excess of the amount excreted in the ordinary single injection experiments.

In these experiments the dog usually became nauseated and vomited following the single injection. The vomitus was a mucoid bile stained fluid and was found to contain uric acid.

DISCUSSION. It is not surprising to find that the decrease in blood uric acid following a single intravenous injection is a logarithmic curve corresponding to a monomolecular reaction. As was mentioned in the introduction, it is now generally believed that in the dog most of the uric acid is oxidized to allantoine. Folin and Berglund have shown that uric acid does not readily diffuse into the general body tissues and Mann and his co-workers have definitely shown that the liver is the sole organ concerned in the oxidation or destruction of uric acid. Since only very small quantities of the uric acid were excreted in the urine, it is very reasonable to suppose that the nature of the blood uric acid curves is indicative of a monomolecular reaction in which one molecule of uric acid isoxidized to one molecule of allantoine.

The results obtained with continuous injections of uric acid are very interesting and show that the ability of the dog to destroy uric acid is sharply limited. When the rate of inflow of uric acid exceeds the maximal rate at which uric acid can be oxidized it accumulates in the blood. With the dog used in these experiments the injection of 10 mgm. of uric acid per minute resulted in a steady state of the blood uric acid level which could be maintained at about 3.3 mgm. per 100 cc. for as long as three hours, thus indicating that the rate of inflow was exceeding the rate of destruction but that both were constant. It is quite likely that different dogs would show differences in the rate of injection necessary to maintain the steady state.

When a single injection was combined with a continuous injection, the decrease of the blood uric acid following the single injection was a logarithmic curve but it failed to return to the predicted basal level as was the case in simple single injection experiments; in most of the experiments it was from 2 to 3 mgm. above the predicted basal level at the end of one and one-half to two hours. This behavior of the blood uric acid is very similar to that observed in the human subject following a single intravenous injection of uric acid in which the blood uric acid often decreases in a logarithmic curve for a short time, but the decrease soon stops and the blood uric acid may then remain above the normal pre-injection level for a considerable period of time. This finding suggests that the difference between the dog and the human may be quantitative rather than qualitative. This problem is being investigated in greater detail at the present time.

SUMMARY

1. Following a single intravenous injection of from 0.640 to 1.20 gram of uric acid, the blood uric acid rose to a high level. The disappearance of the uric acid from the blood followed a logarithmic curve suggesting a monomolecular reaction. The uric acid excreted in the urine was small in amount and varied between 2.7 and 5.5 per cent of the amount injected.

2. When uric acid was injected continuously at a rate of approximately 10 mgm. per minute, the blood uric acid rose for about 45 or 60 minutes and then reached a steady state of approximately 3.3 mgm. per 100 cc. This steady state could be maintained, by continued injection, for as long as three hours. In these experiments the amount of uric acid excreted in the urine was about 8 or 9 per cent of the quantity injected.

3. When a single injection of 0.8 gram was given during the course of a continuous injection, the decrease in the blood uric acid following the single injection was a logarithmic curve but there was a delay in the return to the level maintained by the continuous injection. It was calculated that from 20 to 36 per cent of the amount given in the single injection was excreted in the urine.

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THE EFFECT OF ANOXEMIA ON THE DIGESTIVE MOVEMENTS OF THE STOMACH

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In the course of experiments on the effect of low oxygen tensions on gastro-intestinal motility, we have already reported the effect of anoxemia on hunger contractions (Van Liere and Crisler, 1930). The effects upon the digestive movements of the stomach are given in the present paper. Any relationship between anoxemia and digestive movements will be particularly important in its application to disease associated with low blood-oxygen tensions such as the gastro-intestinal upsets in cardiac disease. But the rising popularity of living at high altitudes as occasioned by aviation has extended the importance of studying anoxemia beyond the scope of disease.

METHODS. Because of the difficulty of recording movements of the well filled stomach by the balloon method we have used the gastrograph. In no animal did we fail to get records by this method but in several cases no record could be obtained with the balloon, presumably because of defects in the transmitting system caused by kinks, etc. We therefore ran just enough by the balloon method to convince us that the two methods run parallel (see fig. 1). The gastrograph method involves opening the abdomen of the animal so that the present experiments, unlike the ones on hunger contractions, were done under light anesthesia. The data were obtained from fourteen dogs fed from fifteen minutes to one hour before anesthetizing. They were quickly etherized and then injected intravenously with 250 mgm. of barbital-Na per kilo. The animals varied in size from 1.3 to 8.1 kilos and were of all ages. The fixed point of the gastrograph was secured to the transverse band on the lesser curvature of the stomach and the movable point to the corresponding region on the greater curvature. The desired oxygen tensions, adjusted by mixing appropriate concentrations of oxygen and nitrogen in an ethylene-oxygen anesthesia machine, were administered to the animal through a modified Kunde muzzle (Kunde, 1923, and Van Liere and Crisler, 1930). Tracings obtained from animals showing skeletal movements were discarded.

RESULTS. Digestive movements were apparent almost always as quickly as the recording apparatus could be adjusted. Whenever the

oxygen tension became lower than 10 per cent there were distinct signs of inhibition in all animals. Some responded to concentrations as high as 12 per cent. The inhibition consisted of a constant decrease in amplitude of contraction with frequently a fall in tone. The changes in tone were

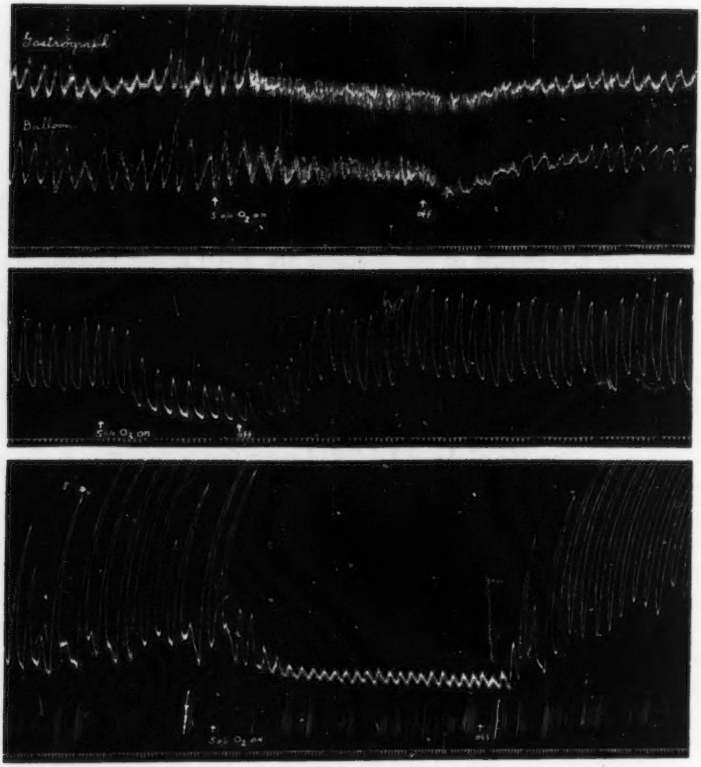


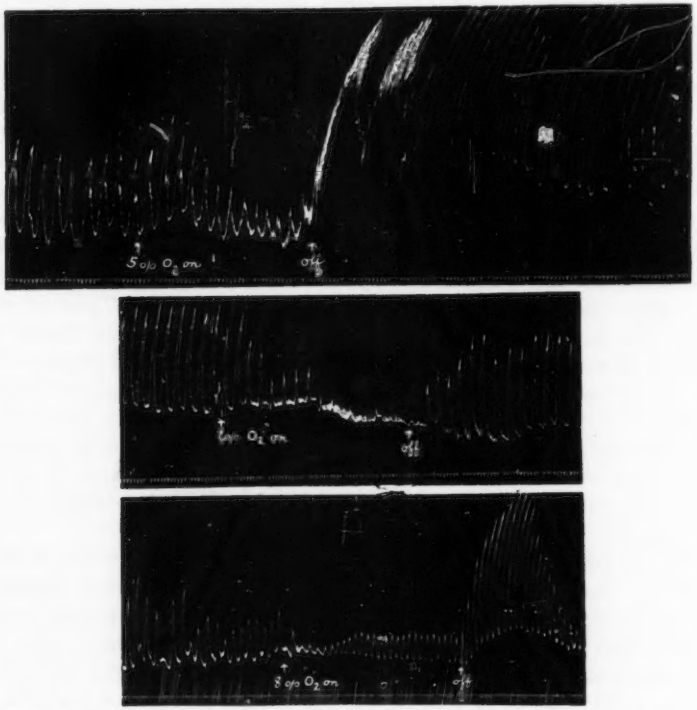
Fig. 1. Simultaneous tracings by gastrograph and balloon to show parallel response by both methods.

Figs. 2 and 3. Response to 5 per cent oxygen in two animals with different amplitudes of contraction and different degrees of tone. Note decrease in amplitude and fall in tone during anoxemia and increase in both to higher than normal levels with stair step effect during recovery.

present when the preceding tone was high, but were absent if the initial tone was sufficiently low (figs. 2 and 6). As the oxygen tension was decreased the inhibition became more nearly complete. In most cases a complete inhibition could be obtained after varying periods with oxygen

tensions below 7 per cent. Occasionally some sort of an "acute tolerance" seemed to develop with a tendency for the contractions to "break through." This was especially noticeable in one animal (fig. 6).

Upon re-oxygenation (allowing the animal to breathe air) there was no post-anoxemic depression as in the case of hunger contractions. The



The similar effect of varying percentages of oxygen below 10 per cent.

Fig. 4. Typical response except for some distortion early in the recovery period caused by forced respiration.

Fig. 5. Typical response except that the original tone is so high that the recovery tone does not surpass it.

Fig. 6. Typical response showing a tendency for the contractions to break through while the tone continues to fall during anoxemia.

recovery usually started immediately. If there was a latent period the contractions were of the same nature as those during anoxemia. The recoveries consisted of prompt contractions of successively greater magnitude giving much the same "stair step" effect as seen after the post-anoxemic depression in hunger contraction experiments. The amplitude almost in-

variably became greater than normal and there was usually a rise in tone to a level higher than normal. In no case were signs of retching or vomiting seen.

Discussion. The liberation of "sympathin" by anoxemia has been suggested as a possible mechanism in these phenomena. In early experiments on sympathin Cannon and Bacq (1931) were unable to show any effect of sympathin on the denervated intestine, and our experiments yield nothing to substantiate such a mechanism.

A far more plausible explanation involving the sympathetics is that in the early period of anoxemia, with the rise in blood pH from hyper-ventilation, the sympathetics are sensitized so that impulses having no inhibitory effect on normal irritability now become effective. Such an increase in the pH of the blood concomitant with the hyperventilation of the early stages of anoxemia has been demonstrated by Koehler, Behneman, Benell and Loevenhart (1925). Burget and Crisler (1927) have shown that such a sensitization of the sympathetics by a rise in blood pH does exist. Such an explanation may well account for the "breaking through" phenomenon also. This sometimes occurs after two or three minutes and agrees well with the duration of the high pH. After this time the pH begins to fall with a release of the sympathetic sensitization. The breaking through may then be simply a partial escape of the intestines from the sympathetic inhibitory effect. If the anoxemia is prolonged an acidosis will eventually occur from the accumulation of fixed acids. The continued inhibition under these conditions of course points to other causative factors as well as alkalosis.

Another possibility is suggested by the work of McSwiney and Newton (1927b). They demonstrated that alkalosis caused a lowered amplitude of contraction in smooth muscle strips. But in previous experiments (1927a) they found a rise in tone under the same conditions. This does not fit with our results where we got decreased amplitude but a fall in tone. We are inclined to think that our results are not based upon the effect of a higher pH directly on the gut musculature.

Gastro-intestinal upsets in certain cardiac diseases especially have been ascribed to the anoxemia accompanying venous stasis. The decreased motility evident in our graphs may help to explain the constipation in such cases but we were unable to reproduce the retching and vomiting. The anesthesia may have prevented their appearance, or the inconsistencies in the two conditions may be accounted for by the fact that in cardiac disease there is relatively rarely a high pH from hyperventilation.

A paradox would appear to exist between the effect of anoxemia on the tone in hunger contractions and in the digestive movements of the stomach. In hunger contractions the tone seemed to rise while in the digestive movements it fell. The difference in the experimental method was only

in the absence of anesthesia in the former case and its presence in the latter. To test the possible effect of anesthesia we have run control hunger contractions (balloon method) on barbitalized dogs. In these cases a fall in tone occurred as in the case of digestive peristalsis. There is a possibility that the increase in gastric tone reported in our hunger contractions was an artifact from less complete relaxation of the diaphram during hyperpnea caused by anoxemia. The apparent rise in tone might come from a transmission of the raised intra-abdominal pressure. A greater hyperpnea with less relaxation of the diaphragm and a higher intra-abdominal pressure might easily occur in the unanesthetized dog but disappear in the anesthetized one. It should be reëmphasized that all records in which skeletal movements were detected were discarded. The amplitude of contraction, however, was decreased during anoxemia in both cases. There is a lack of parallelism in hunger contractions between the experimental results and the clinical picture because there is not a corresponding decrease in the sensation of hunger as might be expected from the decreased amplitude (Carlson, personal communication). On the other hand, there is an apparent parallelism between gastro-intestinal disturbances in the anoxemia of cardiac disease and the effect of anoxemia on gastric digestive motility.

There is no conflict between our results and the appearance of exaggerated peristalsis, "asphyxial peristalsis," as seen in guinea pigs after a fatal blow on the head. Presumably in the guinea pig so treated the pH of the blood falls from the beginning without the preliminary rise, for the animal stops breathing immediately and the factor of initial hyperventilation is absent, hence, assuming the pH as important in the mechanism, the inhibition also would be absent.

A correlation of these inhibitory effects on gastric motility and the emptying time of the stomach under anoxemic conditions will be treated in another paper.

SUMMARY

Anoxemia of grades of 10 per cent oxygen or less, in barbitalized dogs, causes inhibition of gastric digestive motility as indicated constantly by a decreased amplitude of contraction and frequently by a fall in tone.

The most plausible mechanism for the early inhibition seems to be a sensitization of the sympathetics by the rise in blood pH accompanying the initial hyperpnea and hyperventilation in anoxemia.

These results are a partial reproduction of the clinical picture of gastro-intestinal upsets during venous stasis as seen in cardiac disease. A possible explanation of the discrepancies in the two conditions is mentioned.

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THE EFFECT OF SMALL QUANTITIES OF GALACTOSE ON THE HUMAN RESPIRATORY EXCHANGE

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The effect of the ingestion of galactose on the respiratory quotient and the total metabolism has been determined by Lusk (1915) and Wierzuchowski (1931) on dogs and by Deuel (1927) and Cathcart and Markowitz (1927) on men. The amounts of sugar used were 50 and 75 grams with men, and 50 grams and 2 grams per hour per kilo for 3 hours with dogs. As galactose produces a marked change in the respiratory quotient in men with the above quantities, it would seem as though it would be a suitable substance with which to study the effect of varying amounts of sugar on the respiratory quotient. In addition the interest in the metabolism of galactose is increasing, as shown by the number of papers on the effects of ingestion of this sugar. It is well known that there is a lower tolerance for galactose than for either glucose or fructose, and partly for this reason smaller quantities are used than is customary with the other hexoses. Carpenter and Fox (1930a, b) found that there were significant changes in the respiratory quotient and total metabolism after the ingestion of 10 grams or more of either fructose or glucose. We have conducted similar experiments with galactose for the purposes of determining whether the effects upon the respiratory quotient and the total heat production were proportional to the amounts ingested, and of determining how these results compared with those obtained on the influence of the ingestion of glucose and fructose.

METHOD OF DETERMINING THE RESPIRATORY EXCHANGE. The respiratory exchange was determined by means of an open-circuit apparatus which consisted of a helmet (Benedict, 1930), two blowers, a spirometer, the Fox bag method of sampling the air current (Carpenter and Fox, 1931) and two dry gas meters (Benedict and Ritzman, 1931). Samples of air from the Fox bag were drawn through a calcium-chloride tube into mercury samplers or into a metal pump sampler (Benedict and Ritzman, 1931) and later analyzed by an apparatus (Carpenter, Lee, and Finnerty, 1930) designed for the analysis of chamber air. The ventilation through the apparatus was adjusted so that the carbon-dioxide content was approximately 1 per cent. The gas-analysis apparatus was standardized with outdoor air each day.

The average composition of outdoor air in a series of 76 analyses was 0.031 per cent carbon dioxide and 20.940 per cent oxygen, with standard deviations of 0.0017 per cent for carbon dioxide and 0.0038 per cent for oxygen. Usually all of the samples for the 13 or 14 periods were analyzed at least once on the day of the experiment, and then duplicate analyses were made either on the same day or on the day following, particularly on those samples of which the analyses seemed to be aberrant, or those that were of special importance, as for example, a base-line period, the one having the highest respiratory quotient, and the one at the end of the series. The average of the differences in 74 pairs of analyses was 0.005 per cent for carbon dioxide and 0.008 per cent for oxygen. Occasionally analyses of outdoor air were made at the end of the day's series as an additional control.

Procedure of an experiment. The subject was J. C., 48 years, 72.1 kilos, and 167 cm. He arrived at the Laboratory at about 8:30 a.m., urinated, and then sat quietly in a semi-reclining chair for one-half hour, during which his buccal temperature was taken, and records of his previous meal and other data were obtained. The helmet was then adjusted and the ventilation through the apparatus was started. In the experiments in the spring of 1931, four 15-minute periods were run continuously for the base line for the experiment. In the experiments in the fall of 1931, the ventilation was run for 15 minutes without making any records, and then three 15-minute periods were made, as the previous experience had shown that the first 15-minute period was one of adjustment, and therefore was not of value as a base-line measurement. After the base-line periods were finished, the helmet was removed, and the subject rested for 15 minutes to one-half hour, at the end of which the dose for the experiment was given. The sugar was dissolved in 200 cc. of water at 37°C., which the subject drank in as short a time as possible. The helmet was then adjusted, and ten 15-minute periods were run continuously, the helmet was removed, and the subject urinated.

Urine. The statistics of the urinary elimination for the experiments in this group are given in table 1 in which are shown the duration of the period, the volume per hour, the results of the S. R. Benedict (1908) qualitative test, and the amounts of sugar as found by the S. R. Benedict (1911) quantitative method. The subject on arrival at the Laboratory emptied his bladder and then urine was again collected at the end of the experiment, so that there was no separation of periods before and after the ingestion of sugar. Of the total period of time, about 3 to 3½ hours covers the time after the ingestion of the sugar. The volumes per hour averaged highest in the two groups with 30 and 40 grams of galactose. In the other groups the volume is more irregular, although in general the values tend to be nearly as high as those in the three groups mentioned but the excretion is not so regular. In all of the experiments 200 cc. of water were given.

Consequently if there were no need for a water retention, we would expect an excretion which would be equivalent to the amount of water given plus the normal secretion taking place during the given period of time. In one experiment with 5 grams, 2 experiments with 10 grams and one with 20 grams the volume was below the general average. Whether this is due to a retention of water which accompanies the storage of the sugar in the tissues cannot be stated. The high values with the 30 and 40 grams may be due either to a diuresis accompanying the excretion of sugar or to an increased

TABLE 1

Statistics of urine in galactose experiments with Mr. J. C.

DATE	PERIOD	VOLUME PER HOUR	REDUCING SUGAR	
			Qualitative*	Quantitative**
1931		*cc.*		*grams*
5 grams galactose:				
May 2	8:27 a.m.–12:48 p.m.	97	N	
May 4	8:18 a.m.–12:48 p.m.	87	N	
May 8	8:17 a.m.–12:43 p.m.	35	N	
10 grams galactose:				
April 23	8:23 a.m.–12:52 p.m.	77	N	0.7
October 5	6:30 a.m.–12:57 p.m.	15	P	0.7
October 7	8:30 a.m.–12:52 p.m.	31	P	0.6
20 grams galactose:				
March 18	8:38 a.m.– 1:20 p.m.	76	P	
March 23	8:25 a.m.–12:49 p.m.	65		0.9
October 9	8:27 a.m.–12:53 p.m.	35	P	1.1
30 grams galactose:				
March 19	8:33 a.m.–12:53 p.m.	84		2.1
March 24	8:35 a.m.–12:50 p.m.	88		2.2
40 grams galactose:				
March 20	8:32 a.m.–12:49 p.m.	86		3.6
March 25	8:33 a.m.–1:04 p.m.	86		2.5

* N = negative, P = positive.

** Amount of sugar calculated as galactose for the entire period of collection.

excretion of water due to combustion of the sugar. The water due to the combustion of the sugar would amount to 24 grams with 40 grams of galactose.

The excretion of sugar in the urine occurred in all the experiments with 10 grams or over. With the higher amounts of ingested galactose, there were larger amounts of reducing sugars found in the urine, and there is nearly a proportional relationship between the amount of galactose ingested and the amount of sugar in the urine. This subject had a low tolerance for galactose.

The respiratory quotient, combustion of carbohydrates, and heat production after the ingestion of galactose. The respiratory quotients before and after the ingestion of 5 to 40 grams of galactose are given in table 2 for the average of the periods of the base-line hour and for the individual periods following the ingestion of the sugar. The effect of the ingestion of 5 to 40 grams of galactose on the combustion of carbohydrates is shown by the data given by periods in table 3. The changes in heat production caused by the ingestion of varying quantities of galactose are recorded in table 4 for the individual periods. The heat production was calculated from the oxygen

TABLE 2

Respiratory quotients before and after ingestion of galactose (Mr. J. C.)

DATE	BASE LINE	PERIODS AFTER INGESTION OF GALACTOSE									
		1	2	3	4	5	6	7	8	9	10
		0 to 15 min.	15 to 30 min.	30 to 45 min.	45 to 60 min.	60 to 75 min.	75 to 90 min.	90 to 105 min.	105 to 120 min.	120 to 135 min.	135 to 150 min.
1931											
5 grams galactose:											
May 2	0.86	0.85	0.90	0.87	0.83	0.82	0.82	0.81	0.79	0.82	0.80
May 4	0.90	0.91	0.92	0.89	0.88	0.87	0.87	0.87	0.86	0.84	0.85
May 8	0.83	0.83	0.87	0.90	0.87	0.85	0.83	0.83	0.81	0.82	0.81
Average	0.86	0.86	0.90	0.89	0.86	0.85	0.84	0.84	0.82	0.83	0.82
10 grams galactose:											
April 23	0.85	0.81	0.83	0.93	0.89	0.84	0.84	0.83	0.83	0.82	0.80
Oct. 5	0.80	0.80	0.81	0.82	0.80	0.79	0.80	0.79	0.79	0.80	0.80
Oct. 7	0.84	0.84	0.88	0.89	0.88	0.84	0.82	0.83	0.83	0.82	0.83
Average	0.83	0.82	0.84	0.88	0.86	0.82	0.82	0.82	0.82	0.81	0.81
20 grams galactose:											
March 18	0.84	0.83	0.91	0.98	0.97	0.86	0.86	0.80	0.79	0.80	0.80
March 23	0.82	0.82	0.85	0.94	1.01	0.90	0.89	0.85	0.86	0.83	0.84
Oct. 9	0.86	0.84	0.88	0.96	0.96	0.89	0.88	0.87	0.84	0.85	0.83
Average	0.84	0.83	0.88	0.96	0.98	0.88	0.88	0.84	0.83	0.83	0.82
30 grams galactose:											
March 19	0.86	0.86	0.94	0.99	1.01	0.93	0.88	0.86	0.84	0.83	0.81
March 24	0.82	0.91	0.97	0.99	0.98	0.90	0.85	0.84	0.82	0.81	0.81
Average	0.84	0.89	0.96	0.99	1.00	0.92	0.87	0.85	0.83	0.82	0.81
40 grams galactose:											
March 20	0.82	0.81	0.92	0.96	0.99	1.00	0.95	0.90	0.85	0.85	0.83
March 25	0.84	0.87	0.97	1.00	0.99	1.02	0.97	0.91	0.85	0.85	0.85
Average	0.83	0.84	0.95	0.98	0.99	1.01	0.96	0.91	0.85	0.85	0.84

consumption and the respiratory quotient, with an allowance for protein equivalent to a urinary elimination of nitrogen of 0.4 gram per hour. In all three tables, the values were derived from the protocols, which were calculated to one more decimal place than is given in the tables. There

TABLE 3

Carbohydrate metabolism as influenced by the ingestion of galactose (Mr. J. C.)

DATE	BASE LINE*	CHANGES FROM BASE LINE IN PERIODS AFTER INGESTION OF GALACTOSE										
		1	2	3	4	5	6	7	8	9	10	Total change**
		0 to 15 min.	15 to 30 min.	30 to 45 min.	45 to 60 min.	60 to 75 min.	75 to 90 min.	90 to 105 min.	105 to 120 min.	120 to 135 min.	135 to 150 min.	
1931	*grams*	*grams*	*grams*	*grams*	*grams*	*grams*	*grams*	*grams*	*grams*	*grams*	*grams*	*grams*
5 grams galactose:												
May 2	1.83	+0.1	+0.7	+0.1	−0.4	−0.6	−0.4	−0.6	−0.9	−0.6	−0.8	−3.4
May 4	2.39	+0.3	+0.4	−0.1	−0.3	−0.5	−0.5	−0.5	−0.6	−0.9	−0.7	−3.4
May 8	1.23	+0.2	+0.7	+1.0	+0.6	+0.4	+0.2	0.0	−0.1	0.0	−0.1	+3.1
Average	1.82	+0.2	+0.6	+0.3	0.0	−0.2	−0.3	−0.3	−0.5	−0.5	−0.5	−1.2
10 grams galactose:												
April 23	1.67	−0.6	−0.3	+1.3	+0.6	−0.3	−0.3	−0.3	−0.4	−0.3	−0.6	−1.2
Oct. 5	1.10	0.0	+0.2	+0.4	0.0	−0.1	0.0	−0.1	−0.1	0.0	−0.2	+0.1
Oct. 7	1.57	0.0	+0.6	+0.7	+0.5	−0.1	−0.2	−0.2	−0.2	−0.4	−0.2	+0.3
Average	1.45	−0.2	+0.2	+0.8	+0.3	−0.2	−0.2	−0.2	−0.3	−0.2	−0.3	−0.3
20 grams galactose:												
March 18	1.61	−0.2	+1.2	+1.9	+1.7	+0.2	+0.2	−0.5	−0.8	−0.5	−0.6	+2.7
March 23	1.32	0.0	+0.4	+1.5	+1.9	+1.0	+0.8	+0.3	+0.4	+0.1	+0.1	+6.5
Oct. 9	1.70	−0.3	+0.4	+1.5	+1.3	+0.4	+0.2	0.0	−0.3	−0.2	−0.5	+2.4
Average	1.54	−0.2	+0.7	+1.6	+1.6	+0.5	+0.4	−0.1	−0.2	−0.2	−0.3	+3.9
30 grams galactose:												
March 19	1.75	+0.1	+1.6	+1.9	+1.7	+1.2	+0.4	+0.3	−0.1	−0.4	−0.6	+6.1
March 24	1.37	+1.3	+2.3	+2.2	+2.0	+1.1	+0.4	+0.2	−0.1	−0.2	−0.2	+8.8
Average	1.56	+0.7	+2.0	+2.0	+1.9	+1.2	+0.4	+0.2	−0.1	−0.3	−0.4	+7.5
40 grams galactose:												
March 20	1.22	+0.1	+1.8	+2.3	+2.5	+2.3	+2.0	+1.2	+0.6	+0.5	+0.2	+13.5
March 25	1.56	+0.5	+2.3	+2.2	+2.1	+2.0	+1.7	+1.0	+0.2	+0.1	+0.2	+12.2
Average	1.39	+0.3	+2.1	+2.2	+2.3	+2.2	+1.9	+1.1	+0.4	+0.3	+0.2	+12.8

* For 15 minutes.

** For 2½ hours.

are, therefore, some arithmetical discrepancies between the averages and totals given and those found by calculation from the individual values by periods given in the tables.

TABLE 4

Heat production as influenced by the ingestion of galactose (Mr. J. C.)

DATE	BASE LINE*	CHANGES FROM BASE LINE IN PERIODS AFTER INGESTION OF GALACTOSE										
		1	2	3	4	5	6	7	8	9	10	Total change**
		0 to 15 min.	15 to 30 min.	30 to 45 min.	45 to 60 min.	60 to 75 min.	75 to 90 min.	90 to 105 min.	105 to 120 min.	120 to 135 min.	135 to 150 min.	
	cals.	*cals.*	*cals.*	*cals.*	*cals.*	*cals.*	*cals.*	*cals.*	*cals.*	*cals.*	*cals.*	*cals.*
5 grams galactose:												
May 2.....	16.6	+0.5	+0.5	+0.1	+0.6	−0.3	0.0	0.0	+0.2	−0.3	+0.1	+1.4
May 4.....	16.6	+0.4	+0.2	−0.2	−0.6	−0.1	−0.6	−1.0	−0.4	−0.9	−0.3	−3.4
May 8.....	16.1	+0.2	0.0	−0.3	−0.8	−0.8	−0.4	−0.8	−0.1	−0.7	−0.6	−4.3
Average..	16.4	+0.3	+0.2	−0.1	−0.3	−0.4	−0.3	−0.6	−0.1	−0.6	−0.3	−2.1
10 grams galactose:												
April 23....	16.2	−0.5	+0.5	+1.7	+0.1	−0.3	−0.4	−0.5	−1.2	−0.2	+0.2	−0.5
Oct. 5.....	17.2	+0.3	+0.8	+0.3	−0.2	+0.2	+0.2	−0.1	−0.2	−0.2	−0.7	+0.5
Oct. 7.....	16.2	+0.2	+1.0	+0.1	−0.6	−0.8	−0.2	0.0	−0.3	−0.5	−0.1	−1.2
Average..	16.5	0.0	+0.8	+0.7	−0.2	−0.3	−0.1	−0.2	−0.6	−0.3	−0.2	−0.4
20 grams galactose:												
March 18...	16.6	+0.5	+1.8	+1.0	+0.1	+0.1	−0.2	+0.3	0.0	+0.2	−0.1	+3.7
March 23...	15.9	+0.3	+0.8	+0.7	+0.4	−0.5	−0.2	−0.7	−0.4	0.0	−0.2	+0.3
Oct. 9.....	15.5	+0.1	+1.5	+1.0	−0.1	−0.8	−0.9	−1.0	−0.6	−0.7	−1.1	−2.6
Average..	16.0	+0.3	+1.4	+0.9	+0.2	−0.4	−0.4	−0.5	−0.3	−0.2	−0.5	+0.5
30 grams galactose:												
March 19...	15.9	+1.0	+2.7	+2.0	+1.4	+1.3	+1.0	+1.1	+0.7	+0.5	+0.7	+12.4
March 24...	16.3	+1.0	+1.8	+1.4	+0.7	+0.8	+0.2	−0.3	−0.3	−0.2	−0.2	+4.9
Average..	16.1	+1.0	+2.2	+1.7	+1.1	+1.0	+0.6	+0.4	+0.2	+0.1	+0.3	+8.7
40 grams galactose:												
March 20...	15.9	+1.5	+2.0	+2.0	+2.4	+1.7	+1.5	+0.6	+0.5	+0.6	+0.4	+13.2
March 25...	16.1	+1.4	+2.9	+2.3	+1.9	+1.6	+0.4	+0.5	+0.8	−0.2	+0.5	+12.0
Average..	16.0	+1.4	+2.5	+2.1	+2.2	+1.6	+0.9	+0.6	+0.7	+0.2	+0.4	+12.6

* For 15 minutes.

** For 2½ hours.

Five grams of galactose. After the ingestion of 5 grams of galactose there was a rise of 0.02 to 0.05 in the respiratory quotient in the second or third period, even though two of the average base-line quotients were 0.90 and 0.86. The average maximum rise in the 3 experiments was 0.04 in the second period. From there on there was a gradual fall until the end of the series when the quotient was 0.04 below the average base line. There was an increase in the combustion of carbohydrates in the first three periods on the average. The experiment of May 8 differs from the others in that the increase in the combustion of carbohydrate was much larger than in the other two experiments and persists through period 6 (75 to 90 min.). This experiment had the lowest combustion of carbohydrates in the base-line periods, which would lead one to expect a low increase in carbohydrate combustion. There was an increase in the heat production in the first two periods. After the second period, there was a fall in the heat production with three exceptions, periods 3, 4 and 8 of May 2.

Ten grams of galactose. The ingestion of 10 grams of galactose was followed by a maximum rise of 0.08 in the respiratory quotient in the third quarter-hour on April 23 and by an average maximum rise in the 3 experiments of 0.05 in the third period after ingestion. There was a sharp fall in the average quotient from the third to the fifth period after ingestion. The experiment of October 5 differed from the other two in that there was a rise of only 0.02 in the quotient. This experiment was the first of the series in the fall of 1931. The average of 3 observations of buccal temperature on October 5 was 98.7°F., whereas the maximum in any other experiment was 98.1 on April 8. The subject's base-line heat production on October 5 was 17.2 calories per 15 minutes in comparison with a range of from 15.5 to 16.6 calories in all other experiments in this series. He was, therefore, on a distinctly higher plane of metabolism in the experiment of October 5 than in the other two experiments in the group with 10 grams of galactose. There was a definite rise in combustion of carbohydrates in the second and third quarter-hours on October 5 and 7 and in the third and fourth quarter-hours on April 23. After period 4, the values were either nearly those of the pre-ingestion periods or fell below those values. There was a slightly greater increase in heat production than after the ingestion of 5 grams of galactose. The maximum was 1.7 calories in period 3 of April 23, equivalent to 10 per cent of the base line. After the third period, the changes were mostly negative, although not so large as a whole as those in the same periods after the ingestion of 5 grams of galactose. Thus, there was not only a slightly greater increase in the first part of the experiments, but also a smaller fall in the latter part of the experiments in this group than with the preceding group.

Twenty grams of galactose. The ingestion of 20 grams of galactose was followed by a maximum rise of 0.19 in the respiratory quotient in period 4

(45 to 60 min.) on March 23, and by an average maximum rise of 0.14 in the 3 experiments in this same period. There was a rise in the combustion of carbohydrates in periods 2 to 6, inclusive, in all of the experiments and in all of the periods on March 23. The average maximum rise was 1.6 grams in periods 3 and 4, that is, twice as much as with 10 grams of galactose. The last three periods of the group showed about the same fall in combustion of carbohydrates as in the same periods with 10 grams of galactose. There were marked increases in heat production in the second and third periods after ingestion. The maximum rise was 1.8 calories in period 2 of March 18, equivalent to 11 per cent of the base line. The average heat increment in period 2 was 1.4 calories, equivalent to 9 per cent of the average base line.

Thirty grams of galactose. The ingestion of 30 grams of galactose was followed by an average maximum rise of 0.16 in the respiratory quotient in the fourth period (45 to 60 min. after ingestion) of the two experiments, and a rapid fall to the base-line value in the next 3 periods. There was a rise in the combustion of carbohydrates in periods 1 to 7 (90 to 105 min.) in both experiments. The maximum increase in carbohydrate combustion was 2.3 grams in the second quarter-hour on March 24. The maximum rise in heat production was 2.7 calories in the second period (15 to 30 min.) on March 19, equivalent to 17 per cent of the base line. The average rise in this period was 2.2 calories, equivalent to 14 per cent of the base line. In all of the following periods the heat production was, on the average, above the base line, with the minimum average value of +0.1 calorie in period 9.

Forty grams of galactose. The ingestion of 40 grams of galactose was followed by a maximum rise in the respiratory quotient of 0.18 in period 5 (60 to 75 min.) in both experiments, with a rapid fall to a value just above or at the base-line level for the last 3 periods of the experiments. This group was the only group in which the respiratory quotient remained above the base line or pre-ingestion level for two and one-half hours. The greatest rise in combustion of carbohydrates was 2.5 grams in period 4 on March 20 and the greatest average rise was 2.3 grams in period 4. In both experiments the combustion of carbohydrates was greater than the pre-ingestion level throughout all of the periods. There was a rise in heat production in all of the periods in the two and one-half hours of the experiments except period 9 on March 25. The maximum rise in any period was 2.9 calories in period 2 on March 25, equivalent to 18 per cent of the base line.

The ingestion of galactose in quantities varying from 5 to 40 grams was followed by a definite rise in the respiratory quotient. The rise was not directly proportional to the quantity ingested, although each increasingly larger quantity resulted in a successively larger increase. The greatest difference in the rises in quotient between two quantities was 0.09 between

that after 10 grams and that after 20 grams. The period of maximum rise tended to occur about 15 minutes later in each successive group, beginning with period 2 in 5-gram experiments and ending with period 5 in the 40-gram experiments. This differs from the experiments with glucose (Carpenter and Fox, 1930a) in which the maximum quotient appeared 45 to 75 minutes after the ingestion and from the experiments with fructose (Carpenter and Fox, 1930b) in which the maximum quotient was in period 3 (30 to 45 min.) irrespective of the quantity.

The effect on the respiratory quotient of the ingestion of galactose may be estimated in another way, that is, by comparing the average respiratory quotient for two and one-half hours after ingestion with the average base-line quotient. The average of the differences between the average quotient after the base-line hour and that during the base-line hour for each of the different groups is as follows: 5 grams of galactose, −0.006; 10 grams of galactose, −0.001; 20 grams of galactose, +0.031; 30 grams of galactose, +0.051; and 40 grams of galactose, +0.084. Thus there was an increase in the effect of the galactose upon the quotient for two and one-half hours with each succeeding increasing quantity. The average increases per 10-gram steps are more uniform than the maximum rises of quotients over the base line.

The summation of the average increases in combustion of carbohydrates in the successive periods (in which there was an increase) gives the following results for the different groups with galactose: 5 grams, +1.1; 10 grams, +1.3; 20 grams, +4.8; 30 grams, +8.4; and 40 grams, +12.8 grams of carbohydrates. The differences between the successive groups are +0.2, +3.5, +3.6, and +4.4. Thus the largest increase, 4.4 grams, per 10-gram increase in amount given is that between 30 and 40 grams. However, having regard to the relatively small number of experiments in each group, we can tentatively state that for each 10-gram increase in amount of galactose ingested, the additional increase in apparent combustion of carbohydrates was nearly of the same order of magnitude.

When the increases in carbohydrate combustion are expressed as percentages of the amounts of galactose ingested, the values are: 5 grams, 22; 10 grams, 13; 20 grams, 24; 30 grams, 28; and 40 grams, 32 per cent. Thus, with the exception of the 10-gram group, the increase in combustion of carbohydrates in terms of the amount ingested rises slightly and regularly with each successively larger amount of galactose ingested. These values are slightly larger and more regular than those found by Carpenter and Fox (1930a) with glucose and are about the same as those found by the same authors with fructose (Carpenter and Fox, 1930b), but also somewhat more regular than with fructose. The greater regularity may have been due to the use of the helmet, or to the fact that the experiments were

made with the subject in the sitting position, or to a combination of both causes.

The summation of increases in the heat production of the several groups gives the following results: 5 grams galactose, +0.5; 10 grams, +1.5; 20 grams, +2.8; 30 grams, +8.7; and 40 grams, +12.6 calories. Not all of the probable increase in heat production was measured in the 30-gram and 40-gram experiments, especially in the latter group, as the last periods still resulted in increases above the base line. The greatest difference per 10 grams in the increase in the heat production is that between 20 and 30 grams, that is, 5.9 calories. It is probable that the true difference is larger than this, because of the fact that not all of the increase was measured as the experiments did not last long enough.

The stimulating influence on the heat production in relation to the heat of combustion of the ingested galactose, that is, the specific dynamic action, is as follows for the different amounts of sugar: 5 grams, 3; 10 grams, 4; 20 grams, 4; 30 grams, 8; and 40 grams, 8 per cent. Below 20 grams, the specific dynamic action is thus 3 to 4 per cent and above 20 grams rises more rapidly than the increases in amounts given.

The influence of galactose on the heat production was somewhat like that of glucose (Carpenter and Fox, 1930a) in that with the smaller quantities the increases were small, but with the larger quantities, the increases in heat production rose more proportionately than the amounts ingested.

SUMMARY

The effects on the respiratory quotient and the total metabolism of the ingestion of 5 to 40 grams of galactose were determined with a human subject by means of an open-circuit respiration apparatus and a helmet. The base line for the day was measured in three to four 15-minute periods, and ten 15-minute periods were run after the sugar was taken.

The subject had a low tolerance for this sugar as the total reducing substances in urine for approximately 3 hours varied from 0.7 gram after the ingestion of 5 grams to 3.1 grams after the ingestion of 40 grams of galactose.

All amounts of galactose caused a rise in the respiratory quotient, the maximum rise in a period varying from 0.04 with 5 grams to 0.17 with 40 grams. This sugar resembles somewhat fructose in the effects on the quotient, although it differs from both fructose and glucose in that there was a fall to below the pre-ingestion level during the latter part of the two and one-half hours after ingestion.

The rise in apparent carbohydrate combustion varied from 1.1 to 12.8 grams, which represented from 13 to 32 per cent of the amount ingested.

The maximum increase in heat production over the base-line level in a 15-minute period varied from 10 per cent with 10 grams to 18 per cent with 40 grams.

The summation of increases in heat production in successive periods varied from 0.6 calorie with 5 grams to 12.6 calories with 40 grams and the specific dynamic action (the increase in heat production in comparison to the heat of combustion of the sugar) varied from 3 to 8 per cent.

Galactose resembles fructose in its effects on the respiratory quotient and carbohydrate combustion and resembles glucose in its effects on the heat production.

We are indebted to Dr. Allan Winter Rowe of the Evans Memorial Hospital of Boston for providing us with the galactose.

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A COMPARISON OF THE RESPIRATORY EXCHANGE OF MEN AND WOMEN AS AFFECTED BY THE INGESTION OF GALACTOSE

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A difference in metabolism between the two sexes has been found in a number of investigations. The basal metabolism has been observed to be higher in the male with the young human (Benedict and Talbot, 1921); with the adult human (Benedict and Emmes, 1915); with the fowl (Mitchell and Haines, 1927, Mitchell, Card, and Haines, 1927); with the rat (Benedict and MacLeod, 1929); and with the ring dove (Riddle, Christman, and Benedict, 1930). Very recently the last named authors have demonstrated that the variation in heat production according to temperature varies with the ring doves according to sex. Greisheimer (1931) found a materially lower percentage of glycogen in the liver of female rats than that in the liver of male rats after 48 hours of fasting. Deuel and Gulick (1932) noted that a greater ketosis developed in the human female than in the male during fasting. Rowe (1924) found a difference between the sexes in the tolerance for galactose in the human adult in that the average amount of galactose which brought about a S. R. Benedict (1908) qualitative test in the 4-hour urine collected after ingestion was 30 grams with the males, whereas with the females it was 40 grams. Harding and Moberley (1930) confirmed Rowe so far as the qualitative test was concerned, but quantitatively the women excreted more galactose than the men. They offered as a possible explanation the difference in response to the diuretic action of water rather than a real sex difference in the metabolism of galactose. The individual variation was large, so that they stated no definite conclusion could be substantiated. Roe and Schwartzmann (1932) gave 1 gram of galactose per kilo to 5 normal men and 5 normal women and found no evidence for a higher tolerance by women than by men.

While our other studies on the respiratory exchange after the ingestion of galactose (Carpenter and Lee, 1932) were going on, opportunity was offered us by Dr. Allan Winter Rowe to make some observations on members of his staff on the effect of galactose on the respiratory exchange. The apparatus used and the procedure of an experiment were the same as in

the preceding investigation. The average of 68 analyses of outdoor air was 0.031 per cent for carbon dioxide and 20.940 per cent for oxygen, with standard deviations of 0.0014 and 0.0036 per cent, respectively. The average of the differences in 126 pairs of analyses of the experimental samples was 0.004 per cent for carbon dioxide and 0.007 per cent for oxygen. The observations were carried out at the Nutrition Laboratory. Although some of the subjects had had experience with respiration apparatus, none of them had ever worn the helmet (Benedict, 1930) as a breathing appliance. No previous training was given them in the use of the helmet, so that the first experiment recorded with each subject was the first experience.

Statistics of subjects. The statistics of the 5 men and 6 women who acted as subjects are given in table 1. They were all presumably normal.

TABLE 1

Statistics of subjects

SUBJECT	AGE		HEIGHT	WEIGHT WITH CLOTHES
	yrs.	*mos.*	*cm.*	*kilos*
M 1	51	8	165	52.4
M 2	28	9	173	74.9
M 3	35	9	173	86.7
M 4	22	1	186	78.3
M 5	48	0	167	72.1
F 1	21	0	163	51.1
F 2	28	10	164	62.1
F 3	27	5	165	65.5
F 4	23	10	155	51.8
F 5	23	9	170	72.7
F 6	23	10	159	50.7

M-1 is one of the authors (T. M. C.) and M-5 is J. C., the subject of the preceding investigation. The results with M-5 are not included in the averages in tables 3 to 8 because he was the subject in more than one experiment. All weights and ages are the averages for the two experiments in which the subjects were used. F-2 had a weight of 60.1 kilos in 1930 and 64.1 kilos in 1931.

Statistics of urine. The statistics of the urinary elimination in these experiments are given in table 2, namely, the subject, date, period covered, volume per hour, and the results of the S. R. Benedict (1908) qualitative test and the quantitative determination by the S. R. Benedict (1911) method. Unfortunately at the time that the experiments were made, the importance of a careful and rigid examination of the urine for sugar was not recognized, as presumably all of the subjects were normal. Consequently the data are fragmentary and incomplete. Two of the males, M-1

and M-5, gave a positive test with 20 grams; the other three were negative. With 30 grams two, M-2 and M-3, were negative, the same as with 20 grams. M-4 and M-5 were positive. Of the females, four were negative

TABLE 2

Statistics of urine in galactose experiments with men and women

AMOUNT INGESTED	SUBJECT	DATE	PERIOD	VOLUME PER HOUR	REDUCING SUGAR	
					Qualitative‡	Quantitative§
			Men			
grams		*1930*		*cc.*		*grams*
20	M 1	May 26	8:11 a.m.–12:45 p.m.	24	P	0.4
	M 2	July 10	8:20 a.m.– 1:00 p.m.		N	
	M 3	July 11	8:40 a.m.– 1:10 p.m.		N	
	M 4	July 14	8:45 a.m.– 1:17 p.m.	110	N	
	M 5*			59	P	1.0
30	M 2	July 17	8:39 a.m.– 1:03 p.m.	86	N	
	M 3	July 18	8:37 a.m.– 1:08 p.m.	11	N	
	M 4	July 21	8:00 a.m.– 1:18 p.m.	22	P	0.8
	M 5**			86		2.1
			Women			
20	F 1	July 7	1:15 p.m.		N	
	F 2	July 9	8:40 a.m.– 1:05 p.m.		N	
	F 3	July 16	8:20 a.m.–12:55 p.m.	9	N	
		1931				
	F 4	April 14	8:32 a.m.–12:51 p.m.	137	N	
	F 5	April 24	8:33 a.m.– 1:00 p.m.	182	N (?)	
	F 6	May 7	8:53 a.m.– 1:32 p.m.	63	P	0.6
		1930				
30	F 1	July 29	1:10 p.m.		N	
		1931				
	F 2	May 27	8:02 a.m.–12:45 p.m.	111	P	0.5
	F 4	April 28	8:27 a.m.– 1:00 p.m.	125	P	0.8
	F 6	May 14	9:00 a.m.– 1:18 p.m.	24	P	0.8

* Average of 3 experiments. See preceding article, page 635.

** Average of 2 experiments.

‡ N = negative, P = positive, Benedict qualitative test.

§ Amount in total urine voided.

with 20 grams, in one, F-5, the test was uncertain, and F-6 was positive. With 30 grams only one, F-1, was negative out of the four whose urines were tested. The later discussion will show that with F-5 and F-6 the respiratory quotient rose less than with the other females. The data

on the volume are too fragmentary and too limited to discover any relationship between the presence or absence of a qualitative test and the diuresis.

THE RESPIRATORY EXCHANGE OF MEN AND WOMEN AFTER THE INGESTION OF GALACTOSE. *Respiratory quotient.* The respiratory quotients for the base-line period and for the ten 15-minute periods following the ingestion

TABLE 3

Respiratory quotients of men and women before and after the ingestion of 20 grams galactose

SUBJECT	DATE	BASE LINE*	PERIODS AFTER INGESTION OF GALACTOSE									
			1	2	3	4	5	6	7	8	9	10
			0 to 15 min.	15 to 30 min.	30 to 45 min.	45 to 60 min.	60 to 75 min.	75 to 90 min.	90 to 105 min.	105 to 120 min.	120 to 135 min.	135 to 150 min.
Males:	*1930*											
M 1	May 26	0.81	0.82	0.87	0.94	0.92	0.91	0.84	0.81	0.81	0.80	0.79
M 2	July 10	0.78	0.77	0.78	0.85	0.83	0.83	0.82	0.78	0.80	0.80	0.80
M 3	July 11	0.83	0.82	0.94	0.97	0.85	0.81	0.82	0.81	0.81	0.82	0.81
M 4	July 14	0.80	0.78	0.80	0.89	0.93	0.93	0.81	0.77	0.79	0.80	
Average change from base line		0.80	−0.01	+0.04	+0.11	+0.08	+0.07	+0.02	−0.01	0.00	0.00	−0.01
M 5**		0.84	−0.01	+0.04	+0.12	+0.14	+0.04	+0.04	0.00	−0.01	−0.01	−0.02
Females:	*1930*											
F 1	July 7	0.81	0.80	0.86	0.94	0.86	0.81	0.86	0.87	0.82		
F 2	July 9	0.78	0.78	0.83	0.92	1.01	0.82	0.81	0.77	0.76	0.77	
F 3	July 16	0.80	0.79	0.89	1.01	0.91	0.88	0.80	0.81	0.81	0.79	0.81
	1931											
F 4	April 14	0.78	0.77	0.84	0.91	0.86	0.84	0.83	0.82	0.80	0.79	0.79
F 5	April 24	0.82	0.81	0.84	0.87	0.84	0.85	0.82	0.81	0.84	0.81	0.81
F 6	May 7	0.80	0.76	0.78	0.82	0.85	0.83	0.82	0.79	0.81	0.80	0.80
Average change from base line		0.80	−0.01	+0.04	+0.11	+0.09	+0.04	+0.02	+0.01	+0.01	−0.01	0.00

* For 15 minutes.

** Average of 3 experiments. See preceding article, page 635.

of 20 grams of galactose are given in table 3 and for the same periods before and after the ingestion of 30 grams of galactose in table 4.[1] With 20 grams of galactose there was but little change in both sexes in the first period after the ingestion. The average maximum rise above the base-line values was of the same order, namely, 0.11 in the third period (30 to 45 min.) after ingestion. With subject M-5, who was used in the previous investigation, the maximum increase in the respiratory quotient was slightly greater than

[1] The results in tables 3 to 8 are taken from the original protocols which were calculated to the second decimal place. There are some apparent errors in the tables, therefore, when an attempt is made to check the averages or the totals.

with either of the other two groups and occurred in a later period. The fall to the base line after the ingestion took place with both groups in the 7th period (90 to 105 min.) and from there on there is no significant change. There were wide differences in the individual maximum increases in the respiratory quotient in both groups. In the male group, M-2 showed the lowest maximum increase of 0.07, whereas M-1 and M-3 had maximum increases of 0.13 and 0.14 in the third period after ingestion and M-4 had +0.13 in the fourth period after ingestion. None of these are as high as with M-5. With the women the maximum increases were with F-2 in

TABLE 4

Respiratory quotients of men and women before and after the ingestion of 30 grams galactose

SUBJECT	DATE	BASE LINE*	PERIODS AFTER INGESTION OF GALACTOSE									
			1	2	3	4	5	6	7	8	9	10
			0 to 15 min.	15 to 30 min.	30 to 45 min.	45 to 60 min.	60 to 75 min.	75 to 90 min.	90 to 105 min.	105 to 120 min.	120 to 135 min.	135 to 150 min.
Males:	*1930*											
M 2	July 17	0.82	0.80	0.87	0.96	0.96	1.02	0.94	0.91	0.84	0.84	0.84
M 3	July 18	0.83	0.85	0.98	0.99	0.95	0.87	0.84	0.84	0.83	0.82	0.80
M 4	July 21	0.79	0.82	0.92	0.97	0.93	0.84	0.83	0.82	0.79	0.81	0.82
Average change from base line		0.82	+0.01	+0.11	+0.16	+0.13	+0.10	+0.05	+0.04	0.00	+0.01	0.00
M 5**		0.84	+0.05	+0.12	+0.15	+0.16	+0.08	+0.03	+0.01	−0.01	−0.02	−0.03
Females:	*1930*											
F 1	July 29	0.88	0.91	0.95	1.07	1.01	0.94	0.90	0.89	0.91	0.93	0.89
F 3	July 30	0.84	0.82	0.92	1.03	1.04	1.01	0.89	0.86	0.85	0.86	
	1931											
F 2	May 27	0.81	0.81	0.90	0.94	1.00	0.95	0.86	0.82	0.80	0.79	0.79
F 4	April 28	0.80	0.76	0.82	0.93	1.01	0.97	0.86	0.80	0.82	0.83	0.79
F 6	May 14	0.79	0.77	0.80	0.87	0.91	0.92	0.91	0.87	0.85	0.80	0.77
Average change from base line		0.82	−0.01	+0.06	+0.15	+0.17	+0.14	+0.06	+0.02	+0.02	+0.02	−0.01

* For 15 minutes.

** Average of 2 experiments. See preceding article, page 635.

the fourth period (45 to 60 min.) after ingestion, namely, 0.23, and with F-3 in the period preceding, namely, 0.21. The lowest maximum increases were with F-5 and F-6, 0.05 in the third and fourth periods, respectively. There is thus a greater variation in the maximum increases in the respiratory quotient with the women than with the men. The cause for the small increase with M-2 is not known. He had been nearly 24 hours without food whereas the others had abstained from food for only about 12 to 15 hours. There were also individual differences with regard to the rate at which the quotient returned to the base line after ingestion. For example, with M-3 the return occurred in the fifth period after ingestion and from then on the values were below the base line, whereas with M-2 there were

both increases and decreases for the entire experiment. Similarly, with F-1 there was not a uniform decline to base line, but rather a variation, the quotient returning to the base line in the fifth period after ingestion and increasing 0.06 in the seventh period. The quotients of F-2 and F-5 returned to base line in the seventh period, but there were some rises later.

In the series with 30 grams of galactose there were only 3 men on whom single experiments were made. With M-5, two experiments were made, the details of which were reported in the preceding publication. The rise with the males came earlier than with the females. For example, in the second period (15 to 30 min.) after ingestion the rise for the 3 males was 0.11 and for M-5, 0.12, whereas with the 5 females it was only 0.06. The maximum rise reached by the males was 0.16 whereas it was 0.17 with the females. The females continued at nearly the same level in the third, fourth, and fifth periods, whereas the males showed a tendency to fall from the high level immediately after the third period. M-5, to a certain extent, resembled the females. The three males showed individual maximum rises varying from 0.20 with M-2 in period 5 to 0.16 with M-3 period 3 after ingestion. With the five women the variations in the individual maximum rises were from 0.21 to 0.13. Four out of five showed maximum rises of 0.19 or over. F-6, who showed one of the smallest rises with 20 grams, also showed the smallest rise here, namely, 0.13.

In general it would seem that the response of the women, so far as the respiratory quotient is concerned, to the ingestion of 30 grams of galactose was slightly more marked in the majority of cases than with the males and the return to the base line was slightly less and slower than that of the males with the exception of M-5. There is thus not a marked difference, on the average, in the rise of the respiratory quotient after 20 and 30 grams with the two sexes, but the variations in the individual rises are slightly greater with the females than with the males.

Relation between maximum rise in respiratory quotient and body weight. An arrangement of the individual maximum rises in respiratory quotients in the order of the increasing body weights of the subjects shows no relation between body weight and increase in quotient.

Relation between maximum rise in respiratory quotient and sugar in urine. There is but little evidence of any relationship between the maximum rise of the respiratory quotient and the amount or appearance of sugar in the urine as indicated by the Benedict qualitative or quantitative determination. In the 20-gram experiments with men the maximum rise in quotient was with M-5 and M-3, one of whom (M-5) showed sugar in the urine in two experiments, the other none. Similarly, the smallest rise was accompanied by no sugar in the urine. The 30-gram experiments with the males all uniformly gave a large maximum rise in the respiratory quotient (range 0.04) with no sugar or with over 3 grams of sugar in the urine. In the 20-

gram experiments with the women the range in the maximum rise was wide (0.18) but only one (F-6) out of 6 showed any sugar in the urine. She also had nearly the smallest rise in respiratory quotient. With the 30-gram experiments with the females, 3 out of 5 had sugar in the urine, but these 3 included the greatest and smallest rise in the respiratory quotient. The only indication of any relationship between the two is with F-6, who had sugar in the urine in both series and also had the smallest or next to the smallest rise in the respiratory quotient.

TABLE 5

Changes from base line of carbohydrate metabolized with men and women after the ingestion of 20 grams galactose

SUBJECT	DATE	BASE LINE*	PERIODS AFTER INGESTION OF GALACTOSE										
			1	2	3	4	5	6	7	8	9	10	
			0 to 15 min.	15 to 30 min.	30 to 45 min.	45 to 60 min.	60 to 75 min.	75 to 90 min.	90 to 105 min.	105 to 120 min.	120 to 135 min.	135 to 150 min.	Total change**
		gms.	*gms.*	*gms.*	*gms.*	*gms.*	*gms.*	*gms.*	*gms.*	*gms.*	*gms.*	*gms.*	*gms.*
Males:	*1930*												
M 1	May 26	1.02	+0.1	+0.7	+1.4	+1.1	+1.0	+0.3	0.0	0.0	−0.1	−0.2	+4.1
M 2	July 10	1.04	−0.2	0.0	+1.0	+0.7	+0.7	+0.6	0.0	+0.2	+0.2	+0.2	+2.5
M 3	July 11	1.96	−0.1	+2.1	+2.5	+0.2	−0.2	−0.1	−0.3	−0.3	−0.1	−0.3	+4.0
M 4	July 14	1.54	−0.4	+0.1	+1.6	+2.5	+2.2	+0.1	−0.5	−0.1	+0.1		+4.8
Average		1.39	−0.1	+0.7	+1.6	+1.1	+0.9	+0.2	−0.2	−0.1	0.0	−0.1	+3.9
M 5‡		1.54	−0.2	+0.7	+1.6	+1.6	+0.5	+0.4	−0.1	−0.2	−0.2	−0.3	+4.3
Females:	*1930*												
F 1	July 7	1.14	0.0	+0.7	+1.6	+0.6	+0.1	+0.6	+0.7	+0.1			+4.2
F 2	July 9	0.79	+0.1	+0.7	+1.7	+2.4	+0.4	+0.3	−0.1	−0.2	−0.1		+5.3
F 3	July 16	1.05	0.0	+1.2	+2.6	+1.4	+0.9	0.0	+0.1	+0.1	−0.1	+0.2	+6.3
	1931												
F 4	April 14	0.86	−0.1	+0.8	+1.5	+0.8	+0.6	+0.5	+0.4	+0.2	0.0	+0.1	+4.3
F 5	April 24	1.49	+0.1	+0.5	+0.9	+0.4	+0.4	+0.1	0.0	+0.5	−0.1	−0.1	+2.7
F 6	May 7	0.92	−0.3	−0.1	+0.3	+0.6	+0.3	+0.2	−0.1	+0.1	0.0	0.0	+1.4
Average		1.04	0.0	+0.6	+1.4	+1.0	+0.5	+0.3	+0.2	+0.1	−0.1	0.0	+4.0

* For 15 minutes.

** Total change from base line computed from the metabolism for the entire experiment by means of the total oxygen absorption and the average respiratory quotient.

‡ Average of 3 experiments. See preceding article, page 635.

Carbohydrate combustion after the ingestion of galactose. The changes in the combustion of carbohydrates during ten 15-minute periods following the ingestion of 20 grams of galactose are shown in table 5 for 5 males and 6 females. The changes by periods were calculated on the assumption that the changes in respiratory quotient were significant and without reference to the participation of protein in the metabolism. The first of these assumptions may not be warranted entirely because part of the rises in respiratory quotient may be due to the formation of acids in the intermedi-

ary metabolism that drive off carbon dioxide with a subsequent retention to compensate for the extra carbon dioxide given off. For comparative purposes between men and women it is believed that the calculated changes serve the purpose. With reference to the second assumption, as long as the protein metabolism was not definitely known, it does not seem worth while to assume an arbitrary value for the subjects. The omission of taking into account the protein results in values given in these tables which are slightly too high.

Both groups of subjects in the 20-gram series showed a definite rise in the carbohydrate combustion in the second period (15 to 30 min.) with a maximum in the third period after ingestion. The greatest rise in any individual period with males was 2.5 grams with M-3 on July 11 in the third period and M-4, fourth period, July 14. Similarly with the females the maximum rise was 2.6 with F-3 on July 16. There is a greater uniformity in the maximum rises with the males than with the females, the range in the latter being from 0.6 with F-6 to 2.6 with F-3. The carbohydrate combustion fell off with the males to the base line or below in 90 minutes after ingestion, whereas with the females it was 120 minutes after ingestion on the average. Subject M-5, with whom 3 experiments were run in the previous investigation, showed about the same order of changes as the other males with a more marked depression after a return to the base line than in the remainder of the group. The total change in carbohydrate combustion for a period of two and one-half hours is given in the last column of the table and shows a uniform rise with the males with the exception of M-2. The female average was practically the same as that of the males, but there was a greater range in the values, that is, from 1.4 to 6.3 grams. F-5 and F-6 gave decidedly lower total increases in carbohydrate combustion than the other four subjects. Excluding these two, the average of the other four would be slightly higher than the general average for the males.

In table 6 are shown the changes in calculated carbohydrate combustion for the experiments with 30 grams. The males gave a greater increase in the carbohydrate combustion practically all through the periods, beginning with the second period (15 to 30 min.) after ingestion, and this rise was shown by all three of the subjects. M-5 had also similarly a rise in the same periods and nearly as marked as the other 3 males. The maximum rise in any single period was 3.4 with M-4 on July 21. With the females marked changes in increases in carbohydrate combustion are shown in periods 3, 4, 5, during the time of 30 to 75 minutes after ingestion, but the increases were not so great on the whole, nor in any single period, as with the males. F-3 is the only subject whose increase in carbohydrate combustion approaches that of the males. Part of the greater increase with the males must be ascribed to the increase in heat production accompanying

the ingestion of 30 grams of galactose. This is shown in table 8. The total increases in carbohydrate combustion for a period of two and one-half hours are shown in the last column of table 6. The 3 males showed a nearly uniform rise, on the average, 10.5 grams of carbohydrate. Subject M-5 did not have quite so large an increase, only 7.9 grams. Four out of the 5 females had a total increase less than the lowest of the 3 males and the average was 7.8 with a maximum value of 9.8 in the series. There was no apparent decrease in carbohydrate combustion in the latter periods of

TABLE 6

Changes from base line of carbohydrate metabolized with men and women after the ingestion of 30 grams galactose

SUBJECT	DATE	BASE LINE*	PERIODS AFTER INGESTION OF GALACTOSE										Total change**
			1	2	3	4	5	6	7	8	9	10	
			0 to 15 min.	15 to 30 min.	30 to 45 min.	45 to 60 min.	60 to 75 min.	75 to 90 min.	90 to 105 min.	105 to 120 min.	120 to 135 min.	135 to 150 min.	
		gms.	*gms.*	*gms.*	*gms.*	*gms.*	*gms.*	*gms.*	*gms.*	*gms.*	*gms.*	*gms.*	*gms.*
Males:	*1930*												
M 2	July 17	1.46	−0.2	+0.8	+2.1	+2.1	+2.4	+1.6	+1.1	+0.3	+0.3	+0.3	+11.2
M 3	July 18	1.94	+0.6	+2.8	+3.0	+2.2	+0.8	+0.2	+0.1	0.0	−0.1	−0.4	+9.4
M 4	July 21	1.32	+0.6	+2.4	+3.4	+2.5	+0.8	+0.8	+0.5	0.0	+0.4	+0.6	+10.9
Average		1.57	+0.3	+2.0	+2.8	+2.2	+1.3	+0.9	+0.6	+0.1	+0.2	+0.2	+10.5
M 5‡		1.56	+0.7	+2.0	+2.0	+1.9	+1.2	+0.4	+0.2	−0.1	−0.3	−0.4	+7.9
Females:	*1930*												
F 1	July 29	1.73	+0.5	+0.9	+1.5	+1.7	+0.8	+0.4	+0.2	+0.4	+0.6	+0.2	+8.0
F 3	July 30	1.51	−0.1	+1.2	+2.3	+2.3	+2.1	+0.7	+0.4	+0.1	+0.2		+9.8
	1931												
F 2	May 27	1.23	+0.2	+1.3	+1.7	+2.6	+1.8	+0.6	+0.1	−0.1	−0.3	−0.3	+7.7
F 4	April 28	1.02	−0.4	+0.4	+1.5	+2.2	+1.7	+0.6	−0.1	+0.2	+0.4	−0.1	+6.7
F 6	May 14	0.89	−0.2	+0.1	+1.0	+1.4	+1.4	+1.3	+0.9	+0.6	+0.1	−0.2	+6.7
Average		1.28	0.0	+0.8	+1.6	+2.0	+1.6	+0.7	+0.3	+0.2	+0.2	−0.1	+7.8

* For 15 minutes.

** Total change from base line computed from the metabolism for the entire experiment by means of the total oxygen absorption and the average respiratory quotient.

‡ Average of 2 experiments. See preceding article, page 635.

the experiments with the males to compensate for the greater increases in the earlier periods with the males as compared with the females. It is apparent that the two quantities of galactose reacted differently with the two sexes, that is, there was a tendency with the females to have larger increases in the carbohydrate combustion with 20 grams, whereas, on the contrary, with 30 grams, the males had the larger increase in carbohydrate combustion.

Heat production after the ingestion of galactose. The heat production for the base-line period and for the changes in heat production in the ten 15-

minute periods following the ingestion of 20 grams of galactose is shown in table 7 for the males and females. The first three periods in both groups gave an increase in heat production, the most marked being in the second period (15 to 30 min.) after ingestion. There is a striking difference in the changes in the heat production in the first period with the females as compared with the males. Without exception the females reacted with a marked increase in the heat production in this period, whereas only one of

TABLE 7

Changes from base line in the heat production of men and women after the ingestion of 20 grams galactose

SUBJECT	DATE	BASE LINE*	PERIODS AFTER INGESTION OF GALACTOSE										
			1 0 to 15 min.	2 15 to 30 min	3 30 to 45 min.	4 45 to 60 min.	5 60 to 75 min.	6 75 to 90 min	7 90 to 105 min.	8 105 to 120 min.	9 120 to 135 min.	10 135 to 150 min.	Total change**
		cals.	*cals.*	*cals.*	*cals.*	*cals.*	*cals.*	*cals.*	*cals.*	*cals.*	*cals.*	*cals.*	*cals.*
Males:	*1930*												
M 1	May 26	12.2	+0.2	+0.8	+0.7	+0.1	−0.1	−0.3	−0.4	0.0	+0.4	+0.3	+1.7
M 2	July 10	17.4	−0.1	+0.5	+0.5	−0.4	−0.2	0.0	−0.3	−1.1	−0.9	−1.1	−3.4
M 3	July 11	19.7	+1.1	+1.9	+1.4	−1.4	+0.8	+1.2	+0.3	+0.6	+0.6	0.0	+6.8
M 4	July 14	20.4	−0.5	+1.2	+0.6	+1.9	+0.2	−1.2	+0.4	+0.6	+0.7		+3.8
Average		17.4	+0.2	+1.1	+0.8	+0.1	+0.2	−0.1	0.0	0.0	+0.2	−0.2	+2.2
M 5‡		16.0	+0.3	+1.4	+0.9	+0.2	−0.4	−0.4	−0.5	−0.3	−0.2	−0.5	+0.6
Females:	*1930*												
F 1	July 7	13.6	+1.6	+1.0	+1.1	0.0	+1.4	+0.6	+0.3	+0.1			+5.9
F 2	July 9	13.3	+1.1	+1.3	+1.3	+0.3	−0.2	−0.1	+0.3	+0.5	0.0		+4.6
F 3	July 16	13.9	+1.8	+1.1	+1.3	+0.8	+0.1	−0.4	+0.1	−0.3	+0.3	+0.6	+5.6
	1931												
F 4	April 14	14.4	+1.3	+1.2	+0.3	−0.7	−0.7	−0.9	−0.8	−0.7	−1.3	−0.8	−3.2
F 5	April 24	16.2	+2.9	+2.4	+2.1	+1.0	0.0	+0.6	+2.0	+2.4	+0.7	+0.7	+14.8
F 6	May 7	12.2	+1.5	+1.2	+0.8	+1.3	−0.1	0.0	−0.5	−0.2	−0.1	+0.3	+4.4
Average		13.9	+1.7	+1.4	+1.1	+0.4	+0.1	0.0	+0.2	+0.3	−0.1	+0.2	+5.4

* For 15 minutes.

** Total change from base line computed from the metabolism for the entire experiment by means of the total oxygen absorption and the average respiratory quotient.

‡ Average of 3 experiments. See preceding article, page 635.

the males, M-3, gave a significant increase in heat production. Similarly in the following three periods, the increases with the females were slightly greater than with the males. After one hour, there is but little change in either direction for most of the periods with both groups of subjects. The total change in two and one-half hours varied widely in both groups of subjects. With the males, M-2 had a decrease of 3.4 calories in two and one-half hours as compared with the average base-line value for that period of time, whereas M-3 had an increase of 6.8 calories. The average of the 4 men was an increase of 2.2 calories for two and one-half hours. The

changes in the heat production of the females varied from −3.2 to +14.8, a wide range in heat production as the result of the ingestion of 20 grams of galactose. The results show that the heat production of the women varied much more in respect to the ingestion of galactose than did that of the men.

The changes in heat production as the result of the ingestion of 30 grams are given in table 8. With all of the males there was a uniform increase in the heat production in the first 6 periods, that is, one and one-half hours after the ingestion of the galactose. Even in subsequent periods there

TABLE 8

Changes from base line in the heat production of men and women after ingestion of 30 grams galactose

SUBJECT	DATE	BASE LINE*	PERIODS AFTER INGESTION OF GALACTOSE										
			1	2	3	4	5	6	7	8	9	10	
			0 to 15 min.	15 to 30 min.	30 to 45 min.	45 to 60 min.	60 to 75 min.	75 to 90 min.	90 to 105 min.	105 to 120 min.	120 to 135 min.	135 to 150 min.	Total change**
		cals.	*cals.*	*cals.*	*cals.*	*cals.*	*cals.*	*cals.*	*cals.*	*cals.*	*cals.*	*cals.*	*cals.*
Males:	*1930*												
M 2	July 17	15.9	+0.4	+1.2	+1.5	+1.3	+0.5	+0.6	−0.2	+0.1	+0.1	+0.5	+6.1
M 3	July 18	19.5	+2.3	+1.9	+2.2	+1.6	+1.2	+0.4	−0.3	0.0	+1.0	+0.8	+11.2
M 4	July 21	19.5	+1.2	+2.1	+2.5	+1.4	+0.4	+1.4	+0.5	+0.5	+1.4	+1.5	+12.7
Average		18.3	+1.3	+1.7	+2.1	+1.4	+0.7	+0.8	0.0	+0.2	+0.8	+0.9	+10.0
M 5‡		16.1	+1.0	+2.2	+1.7	+1.1	+1.0	+0.6	+0.4	+0.2	+0.1	+0.3	+8.8
Females:	*1930*												
F 1	July 29	12.4	+1.0	+1.1	+1.3	+2.1	+1.2	+1.5	+0.6	+0.9	+0.3	+0.8	+11.1
F 3	July 30	14.0	+1.8	+1.7	+1.9	+2.0	+1.2	+0.6	+1.2	−0.6	−0.2		+9.8
	1931												
F 2	May 27	14.7	+1.9	+1.2	+0.7	+1.2	+0.5	+0.1	−0.4	−0.1	−0.4	−0.2	+4.5
F 4	April 28	13.5	+1.1	+1.4	+0.6	0.0	−0.5	−0.7	−0.8	−0.3	+0.2	0.0	+1.1
F 6	May 14	13.2	+0.6	+0.1	+0.9	+0.5	+0.3	0.0	+0.6	−0.3	−0.1	−0.4	+2.2
Average		13.6	+1.3	+1.1	+1.1	+1.2	+0.5	+0.3	+0.2	−0.1	−0.1	+0.1	+5.7

* For 15 minutes.

** Total change from base line computed from the metabolism for the entire experiment by means of the total oxygen absorption and the average respiratory quotient.

‡ Average of 2 experiments. See preceding article, page 635.

was a slight increase, varying from 0.1 to 1.5 calorie. M-5 showed an increase in heat production somewhat similar to the changes with the other 3 males. With the females there were increases in heat production through the first three 15-minute periods for all of the subjects. Subsequently there were varying results. Subjects F-1 and F-3 continued to show an increase in heat production through the seventh period (90 to 105 min.) after ingestion, whereas the other 3 subjects at this period of time had either returned to base line or else the values were below the base-line heat production. The maximum increase in heat production did not occur with

the group as a whole in the first period as it did with 20 grams. With F-1 and F-3 it was in the fourth period, with F-2 in the first period, and with F-4 in the second. F-6 differs from the others in that the increases in heat production were much lower during the first 3 periods. The total increase in heat production with the males varied from 6.1 to 12.7 calories, with an average of about 9.7 calories for the 4 males. Only 2 of the females showed increases in heat production approaching near these values, namely, F-1 and F-3. The other 3 subjects had low increases in heat production, even lower than occurred with 20 grams. Part of the low values can be ascribed to the negative changes in the last 4 or 5 periods of the experiments. The range is much wider than with the males, namely, from 1.1 to 11.1 with a general average of 5.7 calories, which is a little over one-half the average increase for the males.

The initial rise in energy output with the women may have been due to apprehension of the unknown effect of galactose, as the 20-gram experiments with the women were the first that they underwent. The second experiments were with 30 grams and they may not have reacted in the same way. To be sure, the same was true with the men, but our general impression was that the experiments were not so irksome and boresome to the men as to the women.

SUMMARY

The respiratory exchange was measured with an open-circuit apparatus and helmet for ten 15-minute periods with 5 men and 6 women after the ingestion of 20 grams of galactose in 200 cc. water at 37°C. and with 4 men and 5 women after the ingestion of 30 grams of galactose.

The average maximum rise in the respiratory quotient with 4 men was 0.11 in the third quarter-hour after ingestion, and 0.14 with the fifth subject (3 expts.) in the fourth quarter-hour with 20 grams. The female subjects had the same average rise as the 4 men, but a much greater variability individually. The average maximum rise after the ingestion of 30 grams was 0.16 with the men in the third and fourth quarter-hours and 0.17 with women in the fourth quarter-hour. Four out of 5 women had a maximum rise of 0.19 or over, thus greater than with any of the men.

The average increase in apparent combustion of carbohydrate after the ingestion of 20 grams of galactose was 4 grams with both sexes, but the variation with the females, 1.4 to 6.3 grams, was greater than with the males. The average increase with 30 grams was 9.9 grams with the males and 7.8 grams with the females.

The heat production after the ingestion of 20 grams of galactose increased 1.9 calories with 5 men and 5.4 calories with 6 women. After the ingestion of 30 grams, the heat production was raised 9.7 calories with 4 men and

5.7 calories with 5 women. The individual variations were much greater with the women than with the men.

The most marked differences in the two sexes was the tendency for the majority of the females to have higher maximum rises in respiratory quotient, greater increases in heat production at first after 20 grams, the greater increase in carbohydrate combustion and heat production with the males after 30 grams, and the generally wider deviations individually in all the factors with the women.

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A COMPARISON OF THE EFFECTS ON THE HUMAN RESPIRATORY EXCHANGE OF HEXOSES INGESTED SEPARATELY AND TOGETHER[1]

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Although the effects of simultaneous ingestion of two hexoses upon their absorption, on the height of blood sugar rise, and on the excretion of sugar in the urine have been the subjects of a number of investigations, practically nothing is known or can be predicted regarding the effect of ingestion of two sugars at the same time on the total metabolism, either qualitatively or quantitatively, as measured by the respiratory quotient and the total gaseous exchange, respectively. Deuel (1927) determined in man the respiratory quotient and the specific dynamic action following the ingestion of 37.5 grams of glucose plus 37.5 grams of fructose and compared them with the results obtained from the ingestion of 75 grams of sucrose. He found practically the same results in both cases, but as each hexose was not given in a separate experiment, he was unable to ascribe the rises in quotient and metabolism to either component. The studies of Carpenter and Fox (1930a, b) showed that small quantities of glucose and of fructose (10 grams or more) each produced characteristic changes in respiratory quotient and heat production, and the study of Carpenter and Lee (1932a) demonstrated that the ingestion of small quantities of galactose caused changes in respiratory quotient and total metabolism somewhat like those resulting from the ingestion of fructose. Under conditions of ordinary dietetic practices, these sugars are not given separately but in combinations. It is therefore of practical value as well as academic interest to determine whether each sugar maintains its characteristic reaction qualitatively when ingested together with the other sugars, and whether the effects on the heat production are the same. Are the effects on the respiratory quotient additive when the sugars are given together, that is, do they equal the sum of the effects when they are given separately? Is the resultant change in heat production the same as, greater than, or less than the

[1] A preliminary communication was given at the meeting of the XIV[th] International Physiological Congress, Rome, Sept. 2, 1932. Sunti delle comunicazioni scientifiche. 1932. 48.

sum of the changes in heat production after the ingestion of the sugars separately?

To secure information regarding these problems, several series of experiments were made with a human subject, in which the effects of the sugars, glucose, fructose, and galactose when given separately were compared with the effects when given together. Twenty-gram amounts were used, and first a series of experiments with each hexose alone was made, and then combinations of 20 grams each of glucose and fructose, glucose and galactose, fructose and galactose were ingested. Two experiments with 40 grams of lactose were also made to compare the effect of a disaccharide with the effect of its equivalent amount of hydrolysis products, glucose and galactose. The sugars were dissolved in 200 cc. of water at 37°C., and the same volume of water was used whether 20 or 40 grams of sugar were ingested. The procedure of an experiment and the apparatus used were the same as in the two preceding investigations. (Carpenter and Lee, 1932a, b.)

RESULTS OF EXPERIMENTS. The respiratory quotients determined in these experiments are recorded in table 1. The date and the amount and kind of sugar ingested are given in the first column. In the second column are the base-line respiratory quotients, and in the next ten columns the quotients measured following the sugar ingestion. The last column gives the average change from the base line for the whole experiment of two and one-half hours. The average change from the base line in any one group of experiments is likewise shown for each of the ten periods after sugar ingestion.

The amounts of carbohydrate metabolized or burned in the base-line periods and the changes from the base-line amounts after the ingestion of 20 grams each of glucose, fructose, and galactose and combinations of these sugars are recorded in table 2. These values were calculated from the respiratory quotient and oxygen absorption without correcting for protein. The last column gives the total change from the base-line value for the period of two and one-half hours, calculated not by adding the amounts of the individual changes in periods 1 to 10, but from the total carbon-dioxide output and the total oxygen consumption in two and one-half hours. This method gives a more exact figure than the addition of the values in the ten columns, because it does away with the slight variations due to the reduction of the number of decimal places, and also because in the experiments with combinations of fructose and galactose, there were many periods in which the respiratory quotient was higher than unity. When the calculation of the metabolism of carbohydrates was made by periods, it was assumed that the respiratory quotient was unity or below.

The average heat production in the base-line periods and the changes from the base line in 10 successive 15-minute periods are given in table 3.

In these three tables, for purposes of easy reference, Roman numerals

TABLE 1

Respiratory quotients before and after the ingestion of hexoses and combinations of hexoses (Mr. J. C.)

DATE (1931)	BASE LINE	PERIODS AFTER INGESTION OF SUGARS										
		1 0 to 15 min.	2 15 to 30 min.	3 30 to 45 min.	4 45 to 60 min.	5 60 to 75 min.	6 75 to 90 min.	7 90 to 105 min.	8 105 to 120 min.	9 120 to 135 min.	10 135 to 150 min.	Average change
20 grams glucose (I):												
May 11	0.89	0.87	0.91	0.93	0.93	0.89	0.89	0.89	0.88	0.87	0.87	+0.005
October 14	0.83	0.83	0.84	0.84	0.89	0.89	0.88	0.88	0.86	0.87	0.86	+0.030
October 16	0.82	0.80	0.81	0.84	0.86	0.86	0.84	0.84	0.82	0.82	0.82	+0.015
Average change from base line		−0.01	+0.01	+0.02	+0.05	+0.03	+0.03	+0.03	+0.01	+0.01	0.00	+0.015
20 grams fructose (II):												
March 28	0.80	0.80	0.89	0.91	0.92	0.90	0.87	0.86	0.85	0.85	0.83	+0.065
March 30	0.81	0.82	0.90	0.94	0.88	0.88	0.83	0.81	0.78	0.82	0.80	+0.040
October 12	0.84	0.84	0.97	0.95	0.94	0.87	0.86	0.83	0.83	0.82	0.82	+0.035
Average change from base line		0.00	+0.11	+0.12	+0.10	+0.07	+0.04	+0.01	+0.01	+0.01	0.00	+0.045
20 grams galactose (III):												
March 18	0.84	0.83	0.91	0.98	0.97	0.86	0.86	0.80	0.79	0.80	0.80	+0.020
March 23	0.82	0.82	0.85	0.94	1.01	0.90	0.89	0.85	0.86	0.83	0.84	+0.055
October 9	0.86	0.84	0.88	0.96	0.96	0.89	0.88	0.87	0.84	0.85	0.83	+0.020
Average change from base line		−0.01	+0.04	+0.12	+0.14	+0.04	+0.04	0.00	−0.01	−0.01	−0.02	+0.030
20 grams glucose and 20 grams fructose together:												
April 3	0.87	0.90	1.05	1.00	0.95	0.91	0.97	0.96	0.93	0.92	0.88	+0.075
October 19	0.87	0.87	1.01	0.99	0.89	0.92	0.92	0.91	0.86	0.89	0.87	+0.045
Average change from base line		+0.01	+0.16	+0.13	+0.05	+0.05	+0.08	+0.07	+0.02	+0.03	+0.01	+0.060
(I) + (II)		−0.01	+0.12	+0.14	+0.15	+0.10	+0.07	+0.04	+0.02	+0.02	0.00	+0.065
20 grams galactose and 20 grams fructose together:												
April 1	0.82	0.80	0.99	1.05	0.97	0.90	0.88	0.90	0.82	0.81	0.80	+0.075
April 6	0.88	0.88	1.05	1.02	0.95	0.91	0.88	0.87	0.82	0.81	0.84	+0.025
April 17	0.82	0.83	0.97	1.05	0.97	0.91	0.91	0.89	0.87	0.86	0.85	+0.090
May 13	0.79	0.81	0.97	1.02	0.94	0.90	0.89	0.91	0.82	0.82	0.81	+0.100
Average change from base line		0.00	+0.17	+0.21	+0.13	+0.08	+0.07	+0.07	+0.01	0.00	0.00	+0.075
(II) + (III)		−0.01	+0.15	+0.24	+0.24	+0.11	+0.08	+0.01	0.00	0.00	−0.02	+0.080
20 grams galactose and 20 grams glucose together:												
April 15	0.81	0.81	0.84	0.92	0.96	0.93	0.90	0.86	0.86	0.83	0.83	+0.060
April 21	0.86	0.85	0.92	1.02	1.00	0.96	0.91	0.87	0.86	0.85	0.86	+0.050
April 27	0.83	0.82	0.89	0.96	1.00	0.94	0.88	0.86	0.87	0.85	0.83	+0.060
Average change from base line		−0.01	+0.05	+0.13	+0.15	+0.11	+0.06	+0.03	+0.03	+0.01	+0.01	+0.055
(I) + (III)		−0.02	+0.05	+0.14	+0.19	+0.07	+0.07	+0.03	0.00	0.00	−0.02	+0.050
40 grams lactose:												
April 30	0.87	0.84	0.90	0.97	0.98	0.95	0.93	0.90	0.91	0.89	0.88	+0.045
May 15	0.84	0.84	0.89	0.97	0.98	0.95	0.91	0.90	0.88	0.88	0.87	+0.065
Average change from base line		−0.02	+0.04	+0.12	+0.12	+0.09	+0.06	+0.04	+0.04	+0.03	+0.02	+0.055

TABLE 2

The metabolism of carbohydrates as affected by the ingestion of hexoses and combinations of hexoses (Mr. J. C.)

DATE (1931)	BASE LINE*	CHANGES FROM BASE LINE IN PERIODS AFTER INGESTION OF SUGARS										
		1	2	3	4	5	6	7	8	9	10	Total change**
		0 to 15 min.	15 to 30 min.	30 to 45 min.	45 to 60 min.	60 to 75 min.	75 to 90 min.	90 to 105 min.	105 to 120 min.	120 to 135 min.	135 to 150 min.	
	grams	*grams*	*grams*	*grams*	*grams*	*grams*	*grams*	*grams*	*grams*	*grams*	*grams*	*grams*
20 grams glucose (I):												
May 11	2.5	−0.2	+0.5	+0.8	+0.6	0.0	0.0	−0.1	−0.3	−0.3	−0.3	+0.2
October 14	1.6	+0.1	+0.2	+0.2	+0.8	+0.7	+0.6	+0.6	+0.3	+0.4	+0.4	+3.9
October 16	1.5	−0.2	−0.1	+0.3	+0.6	+0.5	+0.2	+0.2	0.0	−0.1	−0.1	+1.3
Average	1.9	−0.1	+0.2	+0.4	+0.7	+0.4	+0.3	+0.2	0.0	0 0	0.0	+1.8
20 grams fructose (II):												
March 28	1.4	−0.1	+1.3	+1.5	+1.6	+1.3	+0.8	+0.6	+0.5	+0.5	+0.3	+8.5
March 30	1.4	+0.4	+1.4	+2.0	+1.3	+1.2	+0.5	+0.2	−0.3	—	0.0	+7.2‡
October 12	1.7	+0.1	+1.9	+1.5	+1.3	+0.4	+0.2	−0.2	−0.2	−0.3	−0.3	+3.7
Average	1.5	+0.1	+1.5	+1.7	+1.4	+1.0	+0.5	+0.2	0.0	+0.1	0.0	+6.5
20 grams galactose (III):												
March 18	1.6	−0.2	+1.2	+1.9	+1.7	+0.2	+0.2	−0.5	−0.8	−0.5	−0.6	+2.7
March 25	1.3	0.0	+0.4	+1.5	+1.9	+1.0	+0.8	+0.3	+0.4	+0.1	+0.1	+7.6
October 9	1.7	−0.3	+0.4	+1.5	+1.3	+0.4	+0.2	0.0	−0.3	−0.2	−0.5	+2.7
Average	1.5	−0.2	+0.7	+1.6	+1.6	+0.5	+0.4	−0.1	−0.2	−0.2	−0.3	+4.3
20 grams glucose and 20 grams fructose together:												
April 3	2.2	+0.6	+2.0	+2.1	+1.2	+0.5	+1.4	+1.0	+0.7	+0.6	+0 3	+11.5
October 19	2.1	+0.1	+2.2	+2.1	+0.3	+0.6	+0.6	+0.5	−0.2	+0.1	−0 2	+6.8
Average	2.2	+0.3	+2.1	+2.1	+0.7	+0.5	+1.0	+0.7	+0.2	+0.3	+0.1	+9.2
(I) + (II)		0.0	+1.7	+2.1	+2.1	+1.4	+0.8	+0.4	0.0	+0.1	0.0	+8.3
20 grams galactose and 20 grams fructose together:												
April 1	1.6	−0.2	+2.9	+3.1	+2.5	+1.3	+1.0	+1.2	0.0	−0.1	−0.3	+12.7
April 6	2.3	+0.2	+2.1	+2.3	+1.3	+0.6	0.0	−0.2	−0.8	−1.0	−0.7	+5.0
April 17	1.5	+0.3	+2.3	+2.8	+2.3	+1.3	+1.4	+1.0	+0.7	+0.5	+0.4	+13.2
May 13	1.2	+0.3	+2.8	+3.2	+2.2	+1.5	+1.4	+1.7	+0.4	+0.4	+0.2	+14.1
Average	1.7	+0.1	+2.5	+2.8	+2.1	+1.2	+0.9	+0.9	+0.1	−0.1	−0.1	+11.3
(II) + (III)		−0.1	+2.2	+3.3	+3.0	+1.5	+0.9	+0.1	−0.2	−0.1	−0.3	+10.8
20 grams galactose and 20 grams glucose together:												
April 15	1.4	+0.1	+0.6	+1.8	+2.4	+1.8	+1.3	+0.6	+0.6	+0.2	+0.2	+10.2
April 21	2.0	0.0	+1.1	+2.2	+2.3	+1.7	+0.9	+0.2	0.0	−0.2	−0.1	+8.2
April 27	1.7	0.0	+1.0	+2.3	+2.7	+1.7	+0.8	+0.4	+0.5	+0.2	−0.1	+9.2
Average	1.7	0.0	+0.9	+2.1	+2.5	+1.8	+1.0	+0.4	+0.4	+0.1	0.0	+9.2
(I) + (III)		−0.3	+0.9	+2.0	+2.3	+0.9	+0.7	+0.1	−0.2	−0.2	−0.3	+6.1
40 grams lactose:												
April 30	2.2	−0.4	+0.6	+1.9	+1.8	+1.3	+1.0	+0.4	+0.5	+0.1	+0.1	+7.8
May 15	1.8	0.0	+0.8	+2.1	+2.3	+1.8	+1.2	+1.0	+0.6	+0.5	+0.4	+10.8
Average	2.0	−0.2	+0.7	+2.0	+2.0	+1.6	+1.1	+0.7	+0.6	+0.3	+0.2	+9.3

* For 15 minutes.

** Computed from the total oxygen and the average respiratory quotient for the entire 2½ hours after the sugars.

‡ For periods 1–8 and 10.

I to III have been assigned to the three series of experiments with the single sugars, and the average period changes from the base line in the series of experiments with combinations of sugars have been compared with the sum of the average period changes in the experiments with single sugars, designated as "(I) + (II)," and so forth. There are also slight discrepancies between the averages and totals given in the tables because the values were taken from the original protocols that were calculated to more significant figures than are given in these tables.

The experiments continued for 2½ hours after the ingestion of the sugar and in the tables the values are given for all of the periods whether or not there was a positive effect for the 2½ hours. The length of time that an increase above base line was found, varies in each group of experiments, and the number of periods in which minus values are found also varies with each group of experiments. The results may be handled in two ways, that is, the values which are positive and which indicate a rise above the base line may be utilized, or the values for the whole period of time may be employed. In the latter case, negative values come into the discussion as well as positive values.

The duration of 2½ hours was employed because it had been found in previous experiments that for the amounts used most of the effect, if not all, was over in 2½ hours, but it has also been found in previous experiments that when varying amounts of sugars are given the negative values after the rise above the base line is completed are not of the same magnitude with each succeeding increasing amount of sugar. Therefore, in order to make a strict comparison one should take into account not only the rises above the base line, but also the negative values below the base line for the total 2½ hours.

In the following discussion, groups of experiments are compared with one another for the total 2½ hours as well as for the succeeding periods. This is done in order to have all the experiments on a comparable basis as far as the duration is concerned. It, however, brings in the apparently anomalous situation that the sum of the rises in the first few periods of the experiment may be greater than the total net change for the 2½ hours, which includes the negative changes in the later portions of the experiment.

Twenty grams of glucose. The first group of experiments was with 20 grams of glucose. The maximum rise in the respiratory quotient in any single period was 0.06 on October 14 in periods 4 and 5. From period 5 to period 10 there was a gradual fall to the base line. The findings in this group are comparable to those in previous studies with about the same amount of glucose. The maximum rise in carbohydrate metabolized was during the fourth period. Thereafter there was a drop to nearly the base-line value in period 7. The average total change calculated from the whole of the experiments is +1.8 grams. The maximum rise in heat pro-

TABLE 3

The heat production as affected by the ingestion of hexoses and combinations of hexoses (Mr. J. C.)

DATE (1931)	BASE LINE*	CHANGES FROM BASE LINE IN PERIODS AFTER INGESTION OF SUGARS										TOTAL CHANGE**
		1	2	3	4	5	6	7	8	9	10	
		0 to 15 min.	15 to 30 min.	30 to 45 min.	45 to 60 min.	60 to 75 min.	75 to 90 min.	90 to 105 min.	105 to 120 min.	120 to 135 min.	135 to 150 min.	
	cals.	*cals.*	*cals.*	*cals.*	*cals.*	*cals.*	*cals.*	*cals.*	*cals.*	*cals.*	*cals.*	*cals.*
20 grams glucose (I):												
May 11	16.9	+0.4	+1.4	+1.4	+0.6	+0.2	+0.2	−0.7	−0.9	−0.3	−0.4	+1.6
October 14	15.6	+0.9	+0.9	+0.9	+0.2	−0.2	−0.3	−0.1	−0.4	−0.5	+0.1	+1.3
October 16	16.2	+0.3	+0.9	+0.7	+0.3	−0.3	−0.4	−0.5	−0.2	−0.6	−0.6	−0.3
Average	16.2	+0.5	+1.1	+1.0	+0.4	−0.1	−0.2	−0.4	−0.5	−0.5	−0.3	+0.9
20 grams fructose (II):												
March 28	18.1	−0.8	−0.3	−0.3	−0.7	−1.1	−1.7	−2.0	−1.7	−2.3	−1.7	−12.6
March 30	17.1	+2.6	+1.1	+0.9	+2.5	+1.9	+2.5	+2.5	+2.7	—	+2.2	+19.0‡
October 12	16.1	+0.5	+1.2	+0.2	+0.1	−0.2	−0.7	−0.7	−0.6	−0.6	−0.9	−1.8
Average	17.1	+0.8	+0.7	+0.2	+0.6	+0.2	0.0	−0.1	+0.2	−1.5	−0.1	+1.5
20 grams galactose (III):												
March 18	16.6	+0.5	+1.8	+1.0	+0.1	+0.1	−0.2	+0.3	0.0	+0.2	−0.1	+3.8
March 23	15.9	+0.3	+0.8	+0.7	+0.4	−0.5	−0.2	−0.7	−0.4	0.0	−0.2	+0.6
October 9	15.5	+0.1	+1.5	+1.0	−0.1	−0.8	−0.9	−1.0	−0.6	−0.7	−1.1	−2.5
Average	16.0	+0.3	+1.4	+0.9	+0.1	−0.4	−0.4	−0.5	−0.3	−0.2	−0.5	+0.6
20 grams glucose and 20 grams fructose together:												
April 3	17.0	+0.9	+1.1	+1.4	+0.4	−0.5	+0.2	−1.2	−0.8	−0.8	+1.4	+2.6
October 19	15.9	+0.7	+2.2	+2.3	0.0	−0.3	−0.1	−0.4	−0.5	−0.9	−1.1	+2.2
Average	16.5	+0.8	+1.7	+1.8	+0.1	−0.4	0.0	−0.8	−0.7	−0.8	+0.1	+2.4
(I) + (II)		+1.3	+1.8	+1.2	+1.0	+0.1	−0.2	−0.5	−0.3	−2.0	−0.4	+2.4
20 grams galactose and 20 grams fructose together:												
April 1	16.9	+1.3	+2.4	+2.7	+2.2	+1.0	+1.3	+0.5	+0.1	0.0	−0.3	+11.6
April 6	16.8	+1.2	+2.1	+2.8	+1.9	+1.1	0.0	−0.2	−0.7	−1.1	−1.3	+6.0
April 17	16.6	+1.1	+1.4	+1.6	+1.2	+0.8	+0.9	+0.2	+0.1	−0.3	+0.2	+7.6
May 13	17.2	−0.4	+1.5	+1.2	+0.7	−0.3	−0.1	+0.3	−0.2	−0.5	−0.7	+1.8
Average	16.9	+0.8	+1.9	+2.1	+1.5	+0.6	+0.5	+0.2	−0.2	−0.4	−0.5	+6.8
(II) + (III)		+1.1	+2.1	+1.1	+0.7	−0.2	−0.4	−0.6	−0.1	−1.7	−0.6	+2.1
20 grams galactose and 20 grams glucose together:												
April 15	16.4	+0.8	+1.7	+1.8	+1.9	+1.4	+0.9	−0.2	−0.6	−0.6	−0.7	+6.5
April 21	16.5	+1.5	+1.7	+1.6	+2.0	+2.1	+1.3	+0.3	+0.3	−0.4	−0.7	+9.6
April 27	16.6	+1.3	+1.7	+2.8	+2.0	+1.4	+1.0	−0.2	−0.6	−0.4	−1.0	+7.9
Average	16.5	+1.2	+1.7	+2.1	+2.0	+1.6	+1.1	0.0	−0.3	−0.5	−0.8	+8.0
(I) + (III)		+0.8	+2.5	+1.9	+0.5	−0.5	−0.6	−0.9	−0.8	−0.7	−0.8	+1.5
40 grams lactose:												
April 30	16.6	+0.5	+1.3	+2.4	+1.4	+1.3	+0.9	+0.1	+0.1	−1.0	−0.4	+6.7
May 15	16.9	+0.1	+0.6	+1.4	+1.6	+1.6	+1.3	+1.1	+0.3	−0.1	−0.2	+7.7
Average	16.8	+0.3	+0.9	+1.9	+1.5	+1.4	+1.1	+0.6	+0.2	−0.5	−0.3	+7.2

* For 15 minutes.

** Computed from total oxygen and average respiratory quotient for the entire 2½ hours after the ingestion of the sugars.

‡ Total for periods 1-8 and 10.

duction was 1.4 calories (8 per cent of the base-line value of 16.9 calories) in periods 2 and 3 on May 11, and the average maximum rise was 1.1 calories (9 per cent) in period 2. This is a half-hour earlier than the maximum rise in carbohydrate combustion (see table 2, period 4). The ingested sugar would supply the energy for a little more than 1 hour. The sum of the increases in the first 4 periods is 3 calories, i.e., 4 per cent of the heat of combustion of the ingested glucose. The average net change in heat production in two and one-half hours was +0.9 calorie.

Twenty grams of fructose. The maximum rise of 0.13 in the respiratory quotient was in period 2 on October 12 and period 3 on March 30. This corresponds to the time when the maximum increase in respiratory quotient occurred in most of the earlier studies of the effect of the ingestion of fructose. The greatest effect in any single experiment was on March 28, where the return to the base line is not so marked as in the other two experiments. The group as a whole shows a fairly rapid return to base-line level. The average rise in quotient for two and one-half hours was 0.045, three times that after glucose. The maximum average rise in the combustion of carbohydrates was in period 3, and from there on until the end of the experiment there was a gradual fall. The rise is larger than with the same weight of glucose and the average total change in carbohydrate metabolized for the whole experiment is +6.5 grams, over three times that for glucose. The 3 experiments with 20 grams of fructose gave different results in the heat production in the individual experiments. On March 28, the base-line value was the highest of any in the series, and all of the periods after ingestion gave lower heat production than the base line. On the contrary, on March 30, all of the periods after ingestion gave increases in heat production in spite of the fact that the base-line value (17.1 cal.) was the second highest in the series. This experiment has been subjected to a theoretical checking by measuring the total ventilation and calculating the composition of expired air from the base-line values with special reference to the percentage of carbon dioxide, which has been found in earlier experiments of this sort (Carpenter and Fox, 1931) to be nearly constant. The average percentage of the base-line periods was then applied to the measured ventilation, and by means of the respiratory quotient the theoretical oxygen absorption was estimated. The calculated values were close enough to those determined to be able to conclude that the observed values were correct. There is no reason to reject the experiment. One has to assume that the dose produced an unusual reaction or that some unknown condition caused a rise in the metabolism. The experiment on October 12 appears to be nearer to the probable effect of ingestion of fructose than the other two. The summation of the average changes in periods 1 to 5 inclusive is 2.5 calories, 3 per cent of the energy content of the ingested fructose. In a previous series (Carpenter and Fox, 1930b), the

increase in heat production after 21 grams of fructose was 0.8 calorie in one hour, thus lower than the group here studied. Reference to table 2 shows that in spite of the lack of uniformity in the results of the calculated heat production, the changes in carbohydrate combustion were, for the most part, positive and much more uniform than the changes in heat production. The respiratory quotient is the determining element in carbohydrate combustion and not the heat production. The course of the combustion of carbohydrate appears to be independent of the heat production.

Twenty grams of galactose. The three experiments with 20 grams of galactose have been given in a preceding publication (Carpenter and Lee, 1932a) but are here repeated for comparison with the other sugars. The maximum rise in the respiratory quotient was 0.19 in period 4 on March 23. The average maximum rise was 0.14 and was one quarter-hour later than with fructose and the same period of time as with glucose. From this period on until the seventh period there was a sharp drop in the respiratory quotient so that all of the periods after the seventh are below the base line with respect to the respiratory quotient. This change to below the base-line level is different from the changes noted with the other two sugars and would point to a slight compensation for over-ventilation during some of the preceding periods. We have no indication as to the effect of galactose upon the alkali reserve in this group of experiments, but Campbell and Maltby (1928) have found that there was no change in the alkali reserve or in the lactic acid of the blood after the ingestion of galactose. Galactose and fructose resemble one another in the effects on the respiratory quotient more than glucose resembles either one of the other two sugars. The maximum rise in combustion of carbohydrate occurred at the same time in this group as with fructose and was nearly of the same order, but both preceding and following these periods the values are somewhat lower than those of fructose. In the last four periods the average change is negative. The summation of the average increases in heat production in periods 1 to 4 is 2.7 calories, 4 per cent of the energy value of the galactose.

The three sugars, glucose, fructose, and galactose in 20-gram doses, produced about the same amount of increase in heat production and the period of maximum increase was in the second quarter-hour after ingestion. The amounts ingested are probably too small to show a differentiation in effects among the three sugars.

Twenty grams of glucose + 20 grams of fructose. The effects on the respiratory quotient of the ingestion of this sugar combination can best be ascertained by comparing the sum of the increases in the respiratory quotients when the two sugars are given separately with the increases when the two sugars are given together. In periods 1, 2, and 3 there is very little difference between these two sets of figures. The sum of the two produces

a more marked effect upon the respiratory quotient, namely, 0.15, 0.10, and 0.07 in periods 4, 5, and 6, as against 0.05, 0.05, and 0.08 when the sugars are given together. The average change in the respiratory quotient for two and one-half hours was +0.060 in the case of the sugars given together as compared with +0.065, the summation of the effect of the two sugars ingested separately. The maximum rise in combustion of carbohydrates occurred in periods 2 and 3. When the effects of these two sugars given separately are added, periods 2 and 3 give an increase of 1.7 and 2.1, respectively, nearly the same as when given together. The total change when 20 grams of glucose and 20 grams of fructose are given separately is 8.3 grams compared with 9.2 found when they are given together. The maximum increase in heat production was 2.3 calories (15 per cent of the base-line value, 15.9 calories) in period 3 on October 19. In both experiments there were marked increases in the first three periods. The average net change in two and one-half hours was 2.4 calories, a value equal to the sum of the net changes of the same sugars given separately.

Twenty grams of galactose + 20 grams of fructose. There was a marked rise in the respiratory quotient in periods 2 and 3, which exceeded or nearly equalled the sum of the effects of the two sugars given separately, but in period 4 the sum of the effects of the two sugars was twice the effect when given together. Thereafter there was not much difference between the two sets of figures as they both return to a base-line level in period 8. The average change in both conditions was nearly the same. The maximum rise in combustion of carbohydrates occurred in period 3, and there was a return, on the average, to base-line level by period 8. The effects of the ingestion of each of these sugars separately, added together by periods, give values not far from those when the two sugars are given together. The maximum difference is in period 4, where the sum is equal to 3.0 grams as compared with 2.1 when given together and +0.1 in period 7 as compared to +0.9. The sum of the total changes in combustion of carbohydrates when the sugars are given separately is equal to 10.8 grams as compared with 11.3 when they are given together. The maximum increase in heat production was 2.8 calories (17 per cent) in period 3 of April 6, and the average change for the same period was +2.1 calories. The average net change in two and one-half hours was +6.8 calories which is over 3 times the sum (2.1) of the effects of the two sugars given separately.

Twenty grams of galactose + 20 grams of glucose. The next group of experiments was with 20 grams of galactose and 20 grams of glucose, which theoretically are the products of hydrolysis of a nearly equal amount of lactose. A summation of the results obtained when the two sugars are given separately compared with the results when the two sugars are given together shows that there was little difference in the effects on the respiratory quotient. The maximum effect of +0.15 when two sugars are given

together is not so large as the sum (0.19) of the effects of the two sugars separately, but the subsequent period shows a larger rise under the first condition than under the latter and there is a greater after-effect, so to speak, when the two sugars are given together than when they are given separately, and their effects added. The average change in respiratory quotient for two and one-half hours was +0.055 when the sugars were given together as compared with +0.050, the sum when given separately. The maximum change in combustion of carbohydrates was in period 4 with a rapid return to a base-line level in period 9 and the experiments, as a whole, are uniform in all three series. The sum of the effects of 20 grams of galactose and of glucose is not so large when these sugars are given separately as when given together. For the first four periods the changes are practically the same but from there on they are somewhat less when the sugars are given separately than when given together. The total change for the two and one-half hours is 6.1 as compared with 9.2 grams. This comparison shows the greatest difference in combustion of carbohydrates of any of the comparisons which have been made. The maximum increase in heat production was 2.8 calories (17 per cent) in period 3 on April 27. The results in this group as a whole were more uniform than in any of the other groups. The average net change in two and one-half hours was 8.0 calories, over 5 times the sum (1.5 calories) of the effects of these two sugars given separately.

Forty grams of lactose. The last group of experiments was with 40 grams of lactose, which is practically equivalent chemically to the preceding group of experiments. The effect of 40 grams of lactose on the respiratory quotient was slightly less from periods 2 to 5 inclusive, than those of a combination of 20 grams each of glucose and galactose, although the difference is not large. From there on the effect of the lactose is slightly larger than the effect of an equivalent amount of the products of hydrolysis, and the return to the base line is not so rapid. In fact, the individual experiments in each group show greater differences in the changes than the differences between the two groups of averages and consequently we may conclude that the effect upon the respiratory quotient is nearly the same when 40 grams of lactose are given as when 20 grams of galactose and 20 grams of glucose are given. The increases in carbohydrate burned after the ingestion of 40 grams of lactose were practically the same as those when its equivalent in a hydrolyzed condition, namely, 20 grams of glucose and 20 grams of galactose, were given. There is a slightly greater effect in the last portion of the experiment when 40 grams of lactose were given than when its equivalent in hydrolyzed constituents were given. It would appear as though there was a slight delay in the effect on the metabolism when lactose is given, as shown by the smaller change in period 4 and the greater changes in periods 6 to 10, although the differences are not marked.

The total change in the two and one-half hours was 9.3 grams, which is practically the same as with the equivalent sugars in the hydrolyzed condition. The maximum increase in heat production in the experiments with 40 grams of lactose was 2.4 calories in period 3 on April 30. The changes in heat production by periods were not so large with 40 grams of lactose as with the equivalent 20 grams each of galactose and glucose, but the effect was more prolonged as the increase lasted one-half hour longer. The average net change in two and one-half hours was +7.2 calories, nearly as large as with the equivalent amount of glucose and galactose.

The results of the studies of the respiratory quotient show in general that when simple sugars are given together, they are additive in the main in the effects on the respiratory quotient and that therefore each sugar maintains its separate effect on the processes as represented by the respiratory quotient whether given singly or whether given in combinations.

In practically all of the groups the maximum change in carbohydrate occurs with the lower base-line level. For example: In the glucose experiments the increases on October 14 and 16 are somewhat larger than the increases on May 11. Similarly, with 20 grams of fructose the increases on March 28 and 30 are larger than the increases on October 12. On March 23, with 20 grams of galactose there is a much greater increase in carbohydrate catabolized than in the other two experiments. In the group with 20 grams of glucose and 20 grams of fructose, the base-line levels are practically identical, but the increases are different in the two experiments. In the four experiments with 20 grams of galactose and 20 grams of fructose, the maximum change occurs with the lowest base-line value, namely, on May 13, and the smallest change occurs with the highest base-line value, namely, on April 6. The same finding holds true with regard to the other two groups, that is, the lower the base-line combustion of carbohydrate is, the greater is the effect on the combustion of carbohydrate, which results from the ingestion of sugars. The effects hold true regardless of whether it is glucose, fructose, or galactose or whether the combinations of these sugars are used.

This finding is different than would be expected if the respiratory quotients in the base-line condition represent the supply of carbohydrate on hand in the body for combustion. One would expect that the lower the respiratory quotient the smaller would be the rise in the respiratory quotients, and consequently in the combustion of carbohydrates after the ingestion of the sugars. The range in respiratory quotients of the base line is from 0.79 to 0.88. The lower value does not indicate a marked depletion of carbohydrate reserves, but the higher value indicates a larger proportion of the combustion of carbohydrates than one would normally expect in the post-absorptive condition. It is possible that when the respiratory quotients are as high as 0.88 it is an indication of a condition which is

not stable, but one which would subsequently change with the respiratory quotient falling gradually. In that case the effect of the ingestion of sugar would not be so great as when the respiratory quotient is at the lower or more normal level of 0.79 to 0.82. Part of the effect of the ingestion of the sugars on the respiratory quotient and combustion of carbohydrate would thus be neutralized by the subsequent fall in respiratory quotient and combustion of carbohydrates when the initial or base-line quotient was high. A lack of rise in the respiratory quotient and in the combustion of carbohydrates would be more likely to take place if the base-line respiratory quotients were more nearly the value of almost exclusive fat combustion. This would not necessarily apply to all three hexoses as with fructose the metabolism may be one of conversion of sugar to fat instead of supplying the glycogen reserves.

Although the increases in respiratory quotients and the increases in combustion of carbohydrate are additive when two hexoses are ingested together, the heat production after combinations with galactose are ingested is greater than the sum of the changes in heat production when the sugars are given separately. This effect is greater than can be accounted for by 20 grams of galactose alone. It apparently is not due to the fact that the amount of sugar ingested is 40 grams instead of 20, as the 40-gram combination of fructose and glucose produces only the sum of the effects of these given separately. It can not be due to the more rapid absorption of galactose in the presence of other sugars, as previous investigations have shown just the contrary. Cori (1925) found with rats that the rate of absorption of galactose was less when galactose was combined with glucose than when it was ingested alone. Corley (1928) observed that the rise in unfermentable reducing substances in the blood and urine of rabbits after the ingestion of galactose alone was delayed when the galactose was combined with glucose, and that an analysis of the intestinal contents showed a delayed absorption of the galactose when glucose was given at the same time. Bodansky (1923) found that the hyperglycemia and the glycuresis that occurred after the ingestion of galactose in dogs were much diminished when an equal quantity of glucose was mixed with the galactose. The presence of the unabsorbed sugar (galactose) may be the cause of the greater heat production, although there is no evidence that unabsorbed nutrients stimulate heat production. It might be that more of the galactose is changed to lactic acid, which would act as a stimulant to metabolism. Wierzuchowski and Laniewski (1931) found that intravenous injection of galactose was followed by a greater rise in lactic acid of the blood than the injection of glucose. It may be that in the presence of other glycogen formers, less of the galactose is converted to glycogen and more of it is burned and that this transformation requires energy. These suggestions are wholly speculative and it is apparent that other experiments are needed

to find the cause of the greater heat production when galactose is combined with other sugars than when given alone.

SUMMARY

With the helmet open-circuit apparatus a study was made of the respiratory exchange of an adult man before and after the ingestion of 20 grams each of glucose, fructose, and galactose and after various combinations of two sugars in 20-gram amounts, each, during one hour previous to ingestion and for two and one-half hours after ingestion, in 15-minute periods.

Glucose and fructose together, galactose and fructose, and galactose and glucose given together produced nearly the same rise in respiratory quotient as the summation of the rises with the sugars given separately. Forty grams of lactose caused slightly smaller rises than the equivalent 20 grams of glucose and of galactose given together, but the return to the preingestion values in the respiratory quotient was not so rapid with lactose as with glucose and galactose.

When glucose and fructose were given together, the increase in carbohydrate combustion averaged 9.2 grams as compared with 8.3, the sum of the increases when given separately. Galactose and fructose given together produced 11.3 grams increase in carbohydrate combustion as compared with 10.8 when given separately. Galactose and glucose given together brought about an average increase of 9.2 grams as compared with 6.1 when given separately. Forty grams of lactose produced an average increase of 9.3 grams of carbohydrate burned as compared with 9.2 when the two sugars equal to the hydrolysis products were given together.

The results were more uniform in all of the experiments with respect to the increases in respiratory quotient and the increases in carbohydrate combustion than they were with respect to the increases in heat production.

When 20 grams each of glucose and fructose were given together, the increase in heat production was 2.4 calories, equal to the summation of the effects of these sugars given separately. However, when 20 grams each of fructose and galactose were given together, the increase in heat production was 6.8 calories as compared with 2.1 when summated. Similarly, the increase in heat production of galactose and glucose given together was 8.0 as compared with 1.5 calories, the sum of the effects when given separately. Forty grams of lactose also produced an increase of 7.2 calories, thus equal to the increase in heat production of the products of hydrolysis of lactose when given separately.

The giving of 20 grams each of glucose, fructose, and galactose in combinations of two sugars thus produces practically the same increases in the respiratory quotient and in the combustion of carbohydrates as would be found by the addition of their effects when given separately. When galactose was combined with another sugar there was a greater increase in heat

production than would be expected from the summation of the effects of galactose and any other sugar when given separately.

It would appear that the qualitative reactions due to the ingestion of hexoses were the same whether given separately or together, but that some other factor than the changes in the transformations of the carbohydrates played a rôle in bringing about the increases in heat production when galactose was ingested with some other sugar. It is suggested that the cause may be the presence of more unabsorbed galactose in the alimentary tract when other sugars are ingested, or the greater formation of lactic acid in the intermediary metabolism of galactose, or the smaller formation of glycogen when other glycogen formers are available.

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THE DIURNAL CYCLE IN THE LIVER

I. Periodicity of the Cycle, with Analysis of Chemical Constituents Involved

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Forsgren, in 1928, observed in the livers of rabbits a cyclic activity of the glycogen-forming and the bile-secreting functions. Using a microchemical technic, he learned that the secretion of bile alternated with the deposition of glycogen and that these two functions were not regulated merely by nutrition, but were probably correlated in some way with a rhythmic activity in the digestive organs. During the night there was a maximal deposition of glycogen and a minimal formation of bile, and during the day the reverse condition prevailed. Even when there was a continuous supply of food in the stomach there were clear signs of periodicity in the liver, so that the food factor alone did not determine the glycogen content. Forsgren, in 1929, designated these two alternating functions as the assimilatory and the secretory phases of the cycle.

Agren, Wilander and Jorpes (1931) described cyclic changes in the glycogen content of the livers of rabbits, rats, and mice, largely independent of food intake. Glycogen accumulated in the liver during the night and disappeared to some extent the next morning. Similar conditions occurred even in fasting animals. These authors studied the cyclic variations of glycogen of the liver of mice and rats under continuous feeding and during fasting, but did not study changes which ensue following a restricted period of available food and water.

Two years ago one of us (Higgins) studied the changes in the weight of the liver which occurred during fasting, after a single period in which food and water were available, and under conditions in which food was constantly available. Chickens, rats and rabbits were studied at that time. When the ratio of the weight of the liver to the weight of the body was plotted against time after feeding, the curve derived was, in every instance, a bimodal one. When chickens were fed from 9:00 to 11:00 a.m., and food and water thereafter withdrawn, the first mode occurred at 3:00 p.m. when the weight of the liver in grams, for each 100 grams of body weight, had increased from 2.0 to 2.8. The second mode occurred at 1:00 a.m. and the low point occurred somewhat later in the rabbit than in the chicken,

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the first from 5:00 to 6:00 p.m. and the second from 12:00 midnight to 2:00 a.m. No attempts were made at that time to determine the chemical constituents which were involved in the changes in the weight of the liver. Recently, however, a study has been made of these cyclic changes in the weights of the livers of white rats after a single feeding period, and at the same time changes in water, glycogen, protein, and fat were followed by chemical analyses.

METHOD. White rats, from our colony, three to five months of age were trained to eat and drink from 9:00 to 11:00 a.m., for four days preceding the experiment. Food and water were removed from the cages each day at 11:00 a.m. On the day of the experiment twenty-five rats, which had been without food and water for the preceding twenty-two hours were weighed and killed, by severing the cervical vessels, at 9:00 a.m. The livers were removed, exsanguinated as far as possible, wiped dry and weighed. These animals of known body and liver weight were the control rats with which the experimental animals were compared, and chemical determinations were made of the water, glycogen, protein, and fat content of the liver of ten of them.

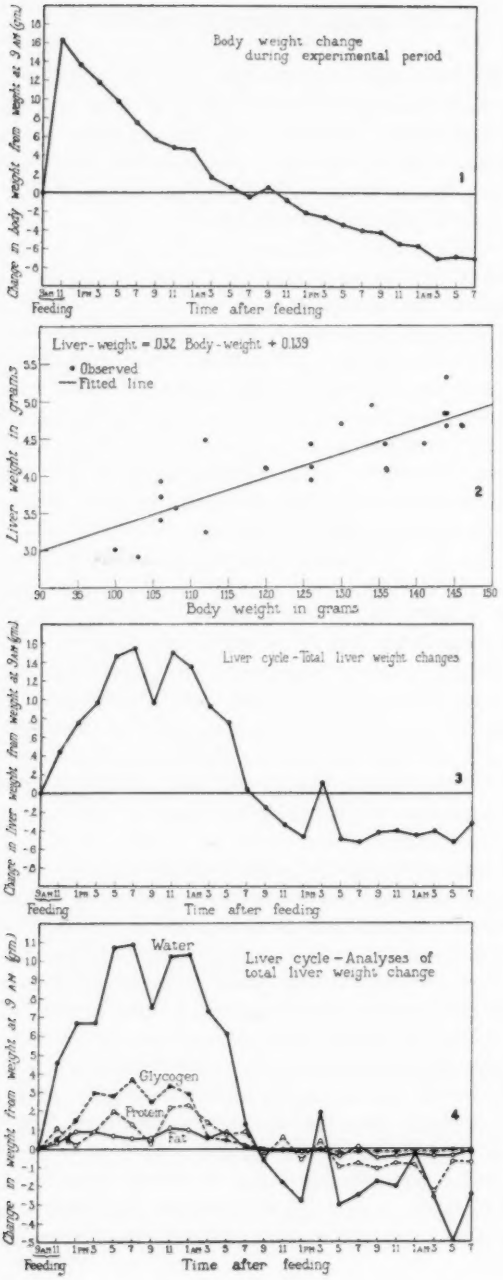
The experimental animals, which had also been fasted for twenty-two hours, were all weighed at 9:00 a.m. and food and water were placed in the cages from 9:00 until 11:00 a.m. At 11:00 a.m. all food and water containers were removed from the cages and beginning at this hour, and at every two-hour interval thereafter for forty-eight hours, six animals were weighed, and killed by severing the cervical vessels. The abdomen was quickly opened, the portion of liver for determination of glycogen was removed and dropped immediately into a freezing mixture of alcohol and carbon dioxide ice. These portions, usually of about 1 gram, were weighed, treated according to the method of Pflüger for glycogen, and the glucose formed was determined by the method of Folin. Other portions of the liver were removed similarly, weighed and analyzed for water, protein and fat. The same region of the liver was selected each time for each particular analysis. For the determinations of water, specimens of liver were dried at a temperature of 85° to 90°F. for a period of seven days, or until constant weight was reached. The loss in weight was determined and considered as water. For the protein analysis the Kjeldahl method for total nitrogen was used, and the value obtained was multiplied by the factor 6.25. The method used for the determination of total fat was essentially that of Bloor. Continuous alcoholic extraction of the samples was followed by the evaporation of the solvent and the extraction of the residue with petrolic ether. The ether soluble substance was dried, weighed, and considered as total fat.

OBSERVATIONS. The mean changes of the weights of the body of all the rats during the two day observation period were computed from their

weight at 9:00 a.m. (fig. 1). The animals gained, on the average, 16.2 grams during the feeding period from 9:00 to 11:00 a.m. A fall in weight was evident immediately thereafter, and this continued without interruption until the end of the experiment. It is of interest that at the end of the first twenty-four hours the mean body weight had returned to about the level recorded at the beginning of the feeding period.

Since the weight of the body was continuously decreasing, it was obviously inexact to express the changes in the liver in terms of a ratio of the weight of the liver to the weight of the body. It appeared preferable to express these changes in terms of the amounts added or lost directly, rather than in terms of a ratio. In order to obtain the change in weight that had taken place at any given hour, since 9:00 a.m. which was taken as the base, it was necessary to have an estimate of the weight of the liver of every experimental animal at 9:00 a.m. To obtain this estimate, we used the weight of the body and the weight of the liver of the twenty-five control rats which had been killed at 9:00 a.m. The weight of the liver was correlated with the weight of the body, and the correlation was found to be linear (fig. 2). A straight line was fitted by least squares to the twenty-five pairs of observations. From the regression equation so obtained (L. W. = 0.032 (B. W.) + 0.139, in which L. W. is weight of liver and B. W. is weight of body) the weight of the liver of each experimental animal at 9:00 a.m. was estimated from its known body weight at 9:00 a.m. Subsequently, the weight of the liver of each experimental animal was determined at the time it was killed, and the difference between the observed weight and that estimated at 9:00 a.m. gave the change in the weight of the liver for that animal. The mean change in the weight of the liver of six animals at each two-hour interval was used as the basis of our calculations.

The experimental findings of the mean changes in the weight of the liver at each two hour interval from the estimated weight at 9:00 a.m., together with their probable errors, have been assembled (table 1). Plus changes denote increases over the weight at 9:00 a.m. while minus changes are decreases from the weight at that time. The curve of these changes in weight (fig. 3) for the first twenty-four hours is definitely bimodal, but is less obviously so in the second twenty-four hour period. The first mode, which indicated the greatest increase in the weight of the liver, occurred at 7:00 p.m. although it was almost as high at 5:00 p.m. The second mode occurred at 11:00 p.m. when the change in weight of the liver recorded was almost as great as that found at 7:00 p.m. At 9:00 p.m., which marked the time of the low point between the two modes, the increase of weight had dropped to 0.968 gram from its previous high point of 1.537 grams. This figure was based on the weights of the livers of nine animals killed at that time.


Body weight change
during experimental period
1
Change in body weight from weight at 9 AM (gms)
Feeding
Time after feeding
Liver - weight = .032 Body - weight + 0.139
• Observed
— Fitted line
2
Liver weight in grams
Body weight in grams
Liver cycle - Total liver weight changes
3
Change in liver weight from weight at 9 AM (gms)
Feeding
Time after feeding
Water
Liver cycle - Analyses of
total liver weight change
Glycogen
Protein
Fat
4
Change in weight from weight at 9 AM (gms)
Feeding
Time after feeding

Figs. 1 to 4

When the value of the mean change at the low point of 9:00 p.m. was compared with those of the modes on either side of it, that is, at 7:00 and 11:00 p.m., the difference in each case was found to be significant statistically. In the first instance, the difference is five times its probable error, and in the second, four times its probable error. Thus the low point 9:00 p.m. reflects a real loss in the weight of the liver from its previous high value, a loss probably associated with physiologic changes correlated with metabolism.

During the second twenty-four hour period the curve of changes in weight of the liver was also bimodal, although the second mode is less clearly defined than the first. All recorded changes, except that at 3:00 p.m., are below the base line and indicate a loss in the weight of the organ from its value at the beginning of the experiment. The mode at 3:00 p.m. is significant statistically, for its probable error is small and the difference between the mean at 1:00 p.m. and at 3:00 p.m. is several times its probable error. The second mode probably occurred at 1:00 a.m., for the mean change in weight of the six animals killed at that time showed a negative change of -0.435 ± 0.168 gram, wherein the probable error was large.

The actual changes which occurred in the water; glycogen, protein and fat content of the liver during the experiment were computed on the same principle as that used in determining the total changes in hepatic weight. The mean percentage content of these constituents of the liver were determined for ten control rats at 9:00 a.m. after a twenty-two hour fast. The following are the percentages found: water 68.7, glycogen 0.82, protein 24.5, and fat 6.9. By multiplying the weight of the liver at 9:00 a.m. by the respective percentage determinations, the total grams of water, glycogen, protein and fat in the liver at 9:00 a.m. were estimated for each experimental animal. At each two-hour interval for the ensuing two day period corresponding percentages were determined, and from these data and the corresponding weights of the liver the amount of each constituent present at that particular interval was computed. The amount estimated at 9:00 a.m. deducted from the amount determined at the time the animals

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Fig. 1. Curve of change of body weight in a series of rats from 9:00 a.m. until 7:00 a.m., two days later. Food and water were withdrawn at 11:00 a.m. the first day.

Fig. 2. Diagram of the weights of liver and weights of body of control rats from which formula for liver weight was derived.

Fig. 3. Curve of change in weight of liver following a single two-hour feeding period, from 9:00 a.m. until 7:00 a.m., two days later.

Fig. 4. Curve of the changes in water, glycogen, protein and fat following a single two-hour feeding period, from 9:00 a.m. until 7:00 a.m. two days later.

were killed gave the change recorded. It is obvious that the calculations do not describe interchanges which most certainly go on continuously in the liver, but give only those net changes which occurred between the intervals.

The mean, with its probable error, and the standard deviations, of the percentage determinations in the chemical analyses of each of the four constituents is given for each interval of the experiment (table 2). During the experiment the percentage of water was relatively constant, ranging from 71.4 per cent at 11:00 a.m. immediately after drinking, to 66.7 per cent at the end of the experiment. The average percentage of water for the 148 animals studied was 69.44. During the first twenty-four hours the glycogen percentage ranged from 0.82 at 9:00 a.m. to 6.510 at 7:00 p.m.,

TABLE 1

Summary of changes in weight of liver: forty-six hours

FIRST TWENTY-FOUR HOURS			SECOND TWENTY-HOURS HOURS		
Time		Change in weight from 9 a.m.	Time		Change in weight from 9 a.m.
Actual	Elapsed		Actual	Elapsed	
	hours	*grams*		*hours*	*grams*
11 a.m.	2	0.449±0.094	9 a.m.	24	−0.157±0.061
1 p.m.	4	0.745±0.139	11 a.m.	26	−0.335±0.099
3 p.m.	6	0.960±0.834	1 p.m.	28	−0.461±0.099
5 p.m.	8	1.474±0.116	3 p.m.	30	+0.108±0.080
7 p.m.	10	1.537±0.053	5 p.m.	32	−0.495±0.094
9 p.m.	12	0.968±0.077	7 p.m.	34	−0.503±0.052
11 p.m.	14	1.498±0.170	9 p.m.	36	−0.416±0.077
1 a.m.	16	1.353±0.079	11 p.m.	38	−0.414±0.033
3 a.m.	18	0.924±0.130	1 a.m.	40	−0.435±0.168
5 a.m.	20	0.750±0.087	3 a.m.	42	−0.411±0.087
7 a.m.	22	0.056±0.090	5 a.m.	44	−0.517±0.075
			7 a.m.	46	−0.324±0.072

eight hours after feeding; a second peak of 5.720 occurred at 11:00 p.m., after which a gradual decline continued until 7:00 the following morning when a glycogen percentage of 0.235 was determined. During the second day the percentage of glycogen varied greatly, the mean for the twenty-four hours being 0.500. Determinations of fat content were remarkably constant during the entire experiment. Percentages ranged between 6.11 and 7.99, the average for the entire series being 6.850. Determinations of protein values varied during the first day from 24.66 to 20.90, the average being 22.96 per cent. The lowest protein percentage occurred at the time of the peak of glycogen, 7:00 p.m. During the second day the percentage of protein was higher and ranged from 24.66 to 30.09, the average being 25.97.

The actual changes in the amount of water and glycogen present in the liver at the time of killing from that estimated at 9:00 a.m. is shown (table 3). The curve of these changes is also included (fig. 4). It is clear that the change in the water content of the liver was the greatest of

TABLE 2

Percentage constituents of liver at various hours after feeding

TIME	WATER		GLYCOGEN		FAT		PROTEIN	
	Mean	Standard deviation	Mean	Standard deviation	Mean	Standard deviation	Mean	Standard deviation
	First twenty-four hours							
	per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
9 a.m.	68.775±0.366	2.10	0.821±0.082	0.47	6.990±0.225	1.00	24.509±0.470	2.70
11 a.m.	71.438±0.138	0.50	1.920±0.105	0.38	6.207±0.082	0.21	24.668±0.427	1.55
1 p.m.	70.938±0.289	1.05	3.578±0.187	0.62	7.290±0.339	0.87	22.680±0.338	1.12
3 p.m.	68.162±0.218	0.79	6.215±0.259	0.94	6.513±0.152	0.39	22.375±0.449	1.63
5 p.m.	69.145±0.245	0.89	5.155±0.050	0.18	6.460±0.078	0.20	22.112±0.333	1.21
7 p.m.	68.620±0.101	0.26	6.507±0.280	0.72	6.990±0.109	0.28	20.903±0.051	0.13
9 p.m.	69.398±0.232	1.03	5.306±0.202	0.90	6.672±0.179	0.65	21.299±0.459	2.04
11 p.m.	68.509±0.531	2.36	5.723±0.413	1.50	7.048±0.094	0.34	21.658±0.380	1.69
1 a.m.	69.943±0.263	1.17	5.460±0.410	1.49	6.875±0.140	0.51	22.744±0.277	1.23
3 a.m.	70.054±0.311	1.03	2.160±0.343	0.72	7.120±0.176	0.37	23.086±0.510	1.69
5 a.m.	69.798±0.223	0.74	1.445±0.329	0.69	7.990±0.005	0.01	23.650±0.509	1.51
7 a.m.	69.708±0.404	1.34	0.235±0.010	0.02	6.780±0.210	0.44	25.935±0.327	0.97
	Second twenty-four hours							
9 a.m.	69.328±0.284	1.03	0.663±0.124	0.45	7.247±0.314	1.14	25.313±0.774	2.81
11 a.m.	69.332±0.039	0.14	0.522±0.036	0.12	7.328±0.306	1.11	30.095±1.809	6.57
1 p.m.	69.128±0.165	0.60	0.468±0.072	0.26	7.020±0.338	1.12	26.573±1.093	3.97
3 p.m.	69.868±0.253	0.92	0.610±0.110	0.40	6.363±0.121	0.44	25.552±0.710	2.58
5 p.m.	68.923±0.121	0.44	0.447±0.069	0.25	6.587±0.135	0.49	25.427±0.606	2.20
7 p.m.	70.565±0.509	1.85	0.455±0.184	0.67	7.894±0.609	2.02	26.082±0.567	2.06
9 p.m.	70.297±0.628	2.28	0.488±0.042	0.14	6.110±0.085	0.31	24.665±0.300	1.09
11 p.m.	70.142±0.438	1.59	0.425±0.030	0.11	6.392±0.127	0.42	25.343±0.363	1.32
1 a.m.	70.188±0.377	1.25	0.578±0.030	0.10	6.794±0.166	0.55	25.514±0.250	0.83
3 a.m.	68.730±0.154	0.51	0.404±0.033	0.11	6.310±0.238	0.79	25.824±0.718	2.38
5 a.m.	69.220±0.235	0.78	0.710±0.148	0.49	6.632±0.181	0.60	26.218±0.489	1.45
7 a.m.	66.718±1.023	3.39	0.266±0.039	0.13	6.830±0.299	0.99	25.074±0.281	0.93

all changes which occurred, and its bimodal curve rather closely follows the curve of the total weight change (fig. 3). The first mode occurred at 7:00 p.m., when the water increase above that present at 9:00 a.m. was 70 per cent of the total weight increase. At 9:00 a.m. on the second day

the water content was slightly below that recorded at the beginning of the experiment, a loss of 0.06 gram having occurred. However, the curve for the second day appears also bimodal, although the changes during this period are not as marked as those which occurred during the first twenty-four hours.

TABLE 3

Summary of composite data

TIME		CHANGE IN WEIGHT FROM 9 A.M.	
Actual	Elapsed	Water	Glycogen
	First twenty-four hours		
	hours	*grams*	*grams*
11 a.m.	2	0.463±0.069	0.0560±0.0052
1 p.m.	4	0.669±0.107	0.1530±0.0109
3 p.m.	6	0.668±0.168	0.3010±0.0330
5 p.m.	8	1.076±0.085	0.2790±0.0072
7 p.m.	10	1.084±0.043	0.3710±0.0206
9 p.m.	12	0.752±0.067	0.2470±0.0148
11 p.m.	14	1.024±0.011	0.3330±0.0441
1 a.m.	16	1.036±0 007	0.2940±0.0242
3 a.m.	18	0.733±0.193	0 0630±0.0215
5 a.m.	20	0.609±0.054	0.5250±0.0253
7 a.m.	22	0.137±0.039	0.0240±0.0029
	Second twenty-four hours		
9 a.m.	24	−0.0603±0.016	−0.0040±0.0055
11 a.m.	26	−0.1801±0.072	−0.0120±0.0010
1 p.m.	28	−0.2888±0.066	−0.0200±0.0173
3 p.m.	30	+0.1938±0.077	−0.0070±0.0055
5 p.m.	32	−0.3030±0.063	−0.0210±0.0019
7 p.m.	34	−0.2490±0.033	−0.0176±0.0036
9 p.m.	36	−0.1801±0.078	−0.0158±0.0017
11 p.m.	38	−0.2048±0.017	−0.0166±0.0010
1 a.m.	40	−0.0388±0.106	−0.0110±0.0033
3 a.m.	42	−0.2614±0.057	−0.0170±0.0009
5 a.m.	44	−0.5070±0.148	−0.0078±0.0051
7 a.m.	46	−0.2524±0.066	−0.0234±0.0009

The curve of the glycogen changes follows fairly well those of the total liver and water, in that the modes occurred at 7:00 p.m., 11:00 p.m. and the low point at 9:00 p.m. The dip in the curve seen at 5:00 p.m. is not significant statistically.

During the second day the glycogen changes were all negative, that is, there was some loss since the beginning of the experiment and their curve is practically a straight line, the livers containing but little glycogen.

Glycogen in the liver appears to be dependent on an available supply of food or water.

The protein changes were also cyclic in their distribution throughout the first twenty-four hours, although the modes did not exactly conform to those of the water and glycogen curves (fig. 4). The first mode occurred at 5:00 p.m. when an increase of 0.201 ±0.030 gram had occurred since 9:00 a.m. and the second mode occurred at 1:00 p.m. when an increase of of 0.230 ±0.011 gram was recorded. The low point between the two was at 9:00 p.m., agreeing in this respect with both the water and glycogen changes. During the second day the determinations showed both increases and decreases from the base level at 9:00 p.m., but a cyclic distribution of these changes in protein did not appear.

COMMENT AND SUMMARY

A study of some of the changes which occurred in the liver of the white rat after a two hour feeding and drinking period is reported. These changes include the actual weight of the liver, as well as the increases or decreases in the amount of water, glycogen, protein and fat for a two-day period after a single feeding.

By means of a formula, determined from statistical data assembled from twenty-five rats, at 9:00 a.m., after a twenty-two hour fast, we were able to estimate the weight of the liver of each experimental rat at that time. During the interval from 9:00 to 11:00 a.m. of the first day of the experiment all rats had access to food and drink. Forced feeding was not attempted, but any animal not freely partaking of food was eliminated from the study. Six animals were killed at every two-hour period and nine at crucial points during the cycle as at 5:00, 7:00, 9:00 and 11:00 p.m. of the first day.

The curve of the changes in the weight of the liver is definitely bimodal. The increase in the weight of the organ continued uninterruptedly until 7:00 p.m.; a significant decrease was encountered at 9:00 p.m. This was transient, however, for the change in the mean weight of the liver at 11:00 p.m. was almost as high as that found at 7:00 p.m. From 11:00 p.m. on a decrease in the total weight was recorded so that at 7:00 the following morning the mean weight of the livers of the six animals killed was essentially that of twenty-four hours before.

We have observed this same bimodal cycle in all animals studied. It occurs in chickens, rabbits and guinea pigs. It may not occur always at the same hour in all animals, but our study has led us to conclude that there are two peaks to the curve of the changes in the weight of the liver during the digestive phase which follows feeding. We have not attempted an explanation to account for the transient decrease in the weight of the liver which occurred at 9:00 p.m. in our series of rats, but it is probably

correlated with the physiologic processes within the liver or perhaps with a rhythmic activity in the gastro-intestinal tract.

During the second day the changes in the weight of the liver were essentially all negative in the sense that the weights were below those estimated at 9:00 a.m. on the first day. A significant rise was encountered at 3:00 p.m. although an immediate decrease recorded at 5:00 p.m. was essentially equal to that recorded at 1:00 p.m. Thus the mode at 3:00 p.m. was comparable, except in extent, to that at 5:00 or 7:00 p.m. the first day. A second mode in the curve of the change in the total weight of the liver was not encountered during the second twenty-four hour period.

It is clear from a study of the curves of the changes in the four constituents of the liver that water, glycogen and to a less extent protein follow rather definitely the curve of the changes in the total weight of the liver. The first mode occurred at 5:00 or 7:00 p.m. and the second occurred at 11:00 p.m. or at 1:00 a.m.; the low point in each case occurred at 9:00 p.m. just as in the curve of the changes in the total weight of the liver. There is little to be said concerning the change in curve for fat, except that it did not follow a bimodal plan such as the changes for water, glycogen and protein, but the increases which were encountered followed more nearly a straight line.

During the second day there did not appear to be any cyclic change, except perhaps in the water content. The marked increase in the water content of the liver at 3:00 p.m. explained the mode encountered at that hour in the curve of the total change in weight. A second mode occurred at 1:00 a.m. but it was considerably less than that at 3:00 p.m. There were practically no changes in the glycogen content during the second day and the determinations were slightly less than those recorded at 9:00 a.m. at the beginning of the experiment. There appeared to be no deposition of glycogen in the livers of these rats in the continued absence of food, even during the night. Furthermore, the determinations of fat and protein largely represented decreases from those recorded at the beginning of the experiment and showed no particular cyclic activity.

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ELECTROMYOGRAPHIC STUDIES OF THE GASTRO-INTESTINAL TRACT

I. The Correlation Between Mechanical Movement and Changes in Electrical Potential During Rhythmic Contraction of the Intestine

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Action currents from the intact musculature of the intestine do not follow the simple forms described in the textbooks as characteristic for skeletal muscle. When recorded they show themselves usually as irregular curves of varying form. Often the records of the electric potential will show a wave-like form which will be more or less regular for a time and in phase with the mechanical contractions of the muscle, but suddenly their appearance will change; the wave form will become irregular, and it will be hard to trace a relationship between the electrical and the mechanical waves.

Recourse to the literature will hardly help clarify matters. Many records of researches on the action potentials of striated and cardiac muscle have been published, and one can find a didactic description of the behavior of electric potentials in any work on the electrocardiogram. The action currents from smooth muscle, on the other hand, have received little attention. From the published work in this field, one obtains the general impression that in interpreting the electrograms one may apply to smooth muscle the same principles that are involved in the electrobiology of skeletal muscle and nerve. When we first undertook this investigation it was with the idea of employing these principles to interpret observed changes of potential in relation to the mechanical movements. Our original plan was merely to continue with an improved technic the studies initiated by Alvarez and Mahoney. We had not proceeded far, however, before it became evident that the relationship between contraction and electric change obtaining in this domain required careful scrutiny, for it presented a number of features not to be expected on the basis of the usually accepted notions regarding action currents in muscle. This fundamental aspect of

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the problem was therefore made the first objective of our investigations. We believe we have made significant progress in that direction, and this report presents the results which we have obtained thus far.

APPARATUS AND EXPERIMENTAL TECHNIC. In order to register simultaneously mechanical contraction and variation in potential we adapted a form of enterograph used by Alvarez so that it should act also as a pair of receiving electrodes. It is pictured to scale in figure 1. The small clip, or serrefine, requires special description. It is made from steel piano wire, 0.018 inch in diameter, bent into the shape shown in the insert of figure 1. Unlike some observers, we have found it impossible to obtain satisfactory results with a polarizable metal electrode, and it was necessary to render the steel serrefine nonpolarizable. This was accomplished by first plating it with silver, and then chloridizing the silver electrolytically.[2] Before use, each pair of electrodes was tested for its nonpolarizable quality.

For most of the investigations reported here, rabbits were used. The animals were anesthetized with urethane or iso-amyl-ethyl barbiturate (amytal) and pithed following the technic described by Alvarez. A median line abdominal incision was made and the intestines were freely exposed.

Action currents may be recorded from the intestines with the animal in the air, or in a warm bath of salt solution but both these arrangements have practical objections. In the air, the animal must be kept warm artificially and the intestine tends to dry, thereby changing the circuit resistance while a record is being taken. In the bath of salt solution the surrounding fluid tends to short-circuit the electrodes and thus to diminish sensitivity. Such a bath tends also to conduct to the electrodes extraneous currents developed here and there in the body. For these reasons an arrangement was made to permit the animal to rest in a warm bath of water, while the intestines floated in inert mineral oil.

It was soon found that any metal coming in contact with the solution of salt would produce electric currents and we therefore had recourse to a

[2] For any who may wish to duplicate this part of the apparatus there are a few practical precautions which must be taken: 1. The steel wire serrefine must be scrupulously clean of fatty covering, and therefore before silver plating it should be boiled in concentrated alkali. For convenience in handling the serrefine during the plating process a short piece of the same type of wire is soldered to the loop. This serves also to lead in the current. 2. Pure silver must be used as the anode in the silver plating process, and for the plating bath, a solution is made up as follows: AgCN, 36 grams per liter, KCN, 52 grams per liter, 1 drop CS_2. 3. The tendency is to silverplate too slowly which gives a rough surface; we use a current density of 10 to 15 milliamperes for each electrode, and plate for three to four hours. 4. In the chloridizing process, the electrode is the anode. A N/2 NaCl or HCl solution is used for the bath, and a low current density, about 1 to 2 milliamperes for each electrode, is applied for about half an hour. 5. In chloridizing, the juncture of the serrefine and the lead-in wire must not be allowed to get into the solution lest the joint forms an electric cell with resultant disintegration.

wooden tank water proofed with wax and varnish. The thermostat and heating elements were all enclosed in glass. The tank was filled to a convenient height with physiologic sodium chloride solution kept at 37°C. and the opened animal was placed into it, its head resting on a canvas support out of the water. A wooden frame about 30 cm. square was then inserted in the tank, and the intestines were allowed to float up into it. The electrodes were now attached to the moistened intestine, and then a quantity of mineral oil sufficient to provide a layer 2 cm. in depth, was poured into the frame to surround the intestines. The tissue to be ex-

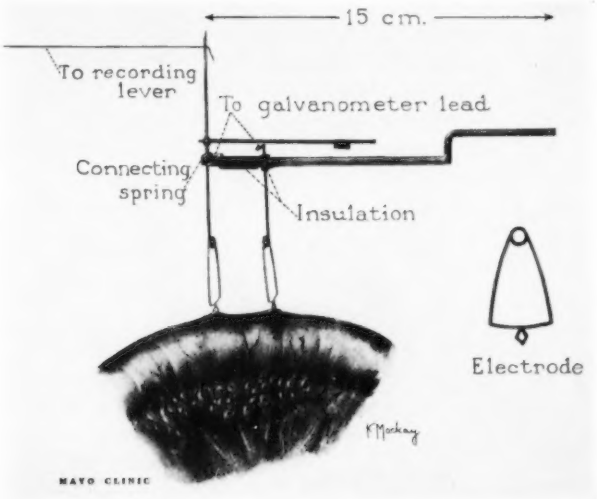

Fig. 1. The apparatus for simultaneous electric and mechanical recording. The electrodes are insulated at the points marked. The connecting spring is made of light copper and is inserted to insure conduction across the joint. The insert shows the serrefine-electrode in detail.

amined was now in an electrically non-conducting medium. The remainder of the body was in a congenial environment which was electrically conducting, a point important for certain of the experiments to be described later. In this state the animal could be maintained for an entire day's observation. At the end of the experiment, the oil was recovered by siphoning.

The changes of electrical potential were recorded by means of a Cambridge string galvanometer, the shadow of the string being photographed on moving bromide paper 12 inches in width. The movement of the intestine was recorded simultaneously on the same paper, by attaching the freely-

swinging electrode with a fine thread to an aluminum lever suspended in the lighted area in front of the camera slit.

RESULTS OF EXPERIMENTS. If a pair of nonpolarizable electrodes are attached to the rabbit's intestine and a simultaneous electrical and mechanical record is made, the results will in general appear as an irregular electric disturbance, with a mechanical record that seems in many instances to have little relation to it. In figure 2 is presented a sample of various records, selected at random from our series.[3] The forms shown are diverse, and still other examples could be multiplied indefinitely. It must be remembered that the intestine commonly shows various types of activity such as pendular movements, tonus waves, and peristaltic rushes,

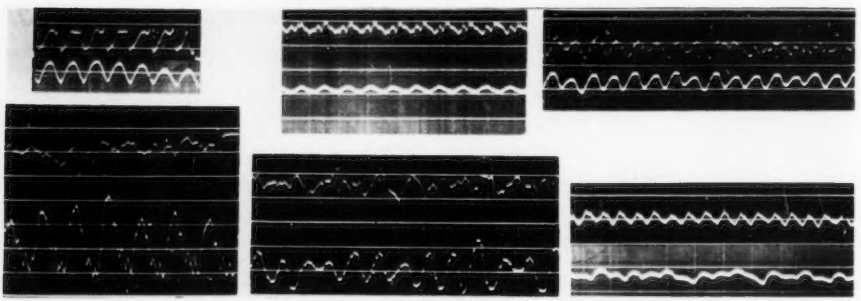

Fig. 2. A sample of various forms of simultaneous electric and mechanical records. The variety could be multiplied indefinitely. In each case the upper record is the electric and the lower is the mechanical. The interval between the time marks is five seconds.

and the rhythmic contractions have different rates in different parts of the intestine. It was reasonable to assume that the varying and irregular form of the electric record, so commonly obtained, was due to the fact that many muscular elements contract with imperfect synchronism and thus contribute many changes of potential to the record. If this were true, we could expect to simplify the record obtained by including smaller amounts of muscular tissue. For this reason we took a very small bite of the serosa with the serrefine-electrode, and attached the electrodes in close approximation to each other, that is, about 1 or 2 cm. apart. These conditions

[3] The electrodes were always attached in a standard way. The one which is free to move back and forth was attached caudally to the fixed one, and the galvanometer was so wired to the electrodes that an upward deflection in the record indicates that the oral electrode has become relatively negative to the caudal one. In the mechanical record an upward deflection corresponds to contraction, and a downward to relaxation.

were adhered to in all the experiments to be described except when otherwise stated.

Doubtless the seeming anarchy of the variations of potential as usually seen is only apparent. If we could analyze completely the multifarious

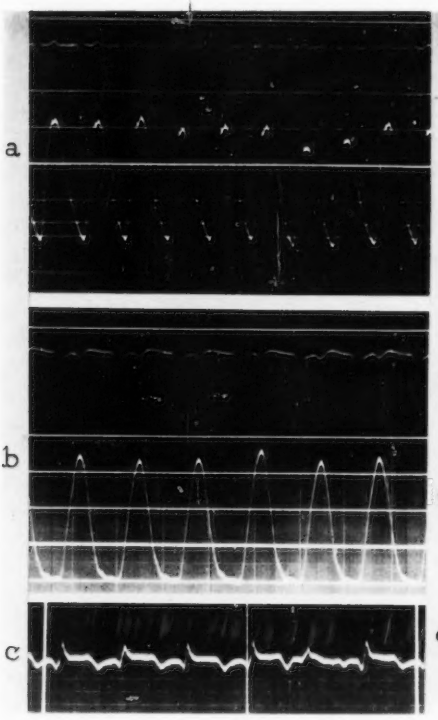

Fig. 3

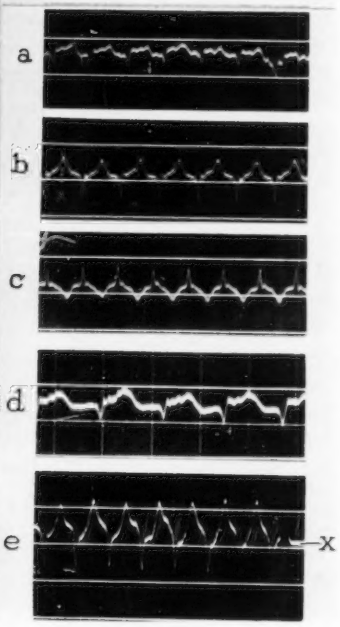

Fig. 4

Fig. 3. a. An example of a characteristic electric wave, with accompanying mechanical record. The upper is the electric, the lower the mechanical record. b. An example of an inverted wave (see text), obtained with bipolar attachment; below it is the simultaneous mechanical record. c. A bipolar record from an isolated segment of a dog's intestine, for comparison (Puestow).

Fig. 4. a. A unipolar record from the oral electrode. b. A unipolar record from the caudal electrode. c. A bipolar record from the same electrodes made in the standard way. The bipolar record is the algebraic difference of the oral and caudal unipolar records. d. A characteristic wave enlarged to show rapid oscillation during the contraction phase. e. To show the presence of a positive variation during the course of rhythmic contraction. The line at the right, x, is the level of zero potential relative to the bath. A deflection below the line indicates a positive change in potential.

activities of the intestine, the record of electric potentials would probably also be clarified. To simplify the analysis somewhat we are reporting only on records obtained from intestine which was executing regular rhythmic contractions. Under these circumstances more regular records are obtained, but they are still far from uniform. In many cases the records are complicated because the electrodes pick up currents that arise in movements of respiration. In what follows we shall describe in some detail a particular form of wave, which we have been able to analyze more successfully than any other. In doing so we do not wish to convey the impression that we have made a complete analysis of the electromyographic record. Much more work must yet be done before this can be effected. The type of electrogram which we here describe appears about once in every ten records taken at random along the intestine, and when seen in its characteristic form it has a definite relationship to the mechanical record.

Both the electric and mechanical records show clearly-defined wave forms, which have the same rate (fig. 3a). The beginning of the wave of contraction, however, does not coincide in time exactly with the beginning of the corresponding electrical wave, but occurs somewhat later. The interval varies in our records and it is most easily accounted for as lag due to the inertia of the mechanical system of recorders. If we align the beginnings of two corresponding waves, a simple relationship over their whole course is apparent. The contraction phase of the mechanical record corresponds exactly to the first half of the electric wave, and the relaxation phase exactly to the second half. These relations are shown more clearly in figure 5, which outlines what we believe to be the correlation of the electric and mechanical phases of rhythmic contraction. The electric wave is seen to consist of two parts. The first half of the wave is a deflection in the upward direction, the second half in the downward direction. The second half is a mirror image of the first. The first part corresponds in time to contraction, the second to relaxation; in other words, the observed electric changes during relaxation are the same as those during contraction, but they occur in the reverse order.

Our accumulated records are illustrative of another striking feature. There are a large number in which the same form of wave appears in the same time relation to the mechanical record, but the entire wave is inverted, that is, the initial deflection in its varying amplitude is in the downward or positive direction (fig. 3b). The record is such as one would expect to obtain by reversing the leads to the galvanometer. It is to be remembered that for all these records, not only those that give the upright type of wave but also those that give the inverted type, the fixed and the movable electrodes bear the same relationship to the oral-caudal direction of the intestine, and that the galvanometer string always deflects upward when the oral attachment is becoming negative relative to the caudal.

Why, then, does the oral attachment sometimes become relatively negative, and sometimes relatively positive to the caudal?

For a while, the explanation was sought in some condition of the intestine, or in the part of the intestine from which the record was obtained. But the question was resolved very simply when a different type of experiment was performed. Instead of connecting the galvanometer to both electrodes that are attached to the intestine, we attached only one; the other was connected with a neutral or standard nonpolarizable electrode, immersed in the bath in a corner of the tank at a distance from the body of the animal. The galvanometer was connected in such a way that an upward deflection indicated that the electrode on the intestine was negative relative to the inactive standard electrode.

When unipolar records were thus taken, the initial deflection was always directed upward, that is, in the negative direction. It is at once clear how

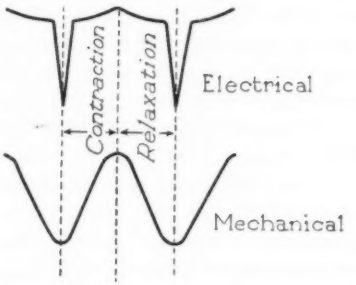

Fig. 5. Diagrammatic illustration of the relationship of the phases of the electric wave to those of the mechanical contraction, when the beginnings of both waves are made to coincide.

an inversion of the wave occurred in the bipolar records. At each electrode the typical wave of the upright type was produced. Since the electrodes were reasonably close, the mechanical contraction was generally in the same phase at both points, and the electric waves were generally identical except for amplitude. If it happened that the wave at the caudal electrode had the larger amplitude the bipolar record would be reversed, for the galvanometer registers the difference in potential between the leads, and it is to be recalled that under the arrangements of the experiments, it was the caudal electrode of the bipolar records that replaced the standard of the unipolar.

That this is the explanation of the appearance of the inverted form of the wave is easily demonstrated experimentally. In figure 4 a, b, and c are shown, first, two unipolar records, one from the oral, the other from the caudal electrode; both are typical wave forms in the normal direction, al-

though that from the oral electrode is not so sharply defined on account of disturbances due to respiratory movements. When the electrodes are attached to these same points for a bipolar record in the regular way, the algebraic difference of these is obtained, and since the caudal wave is of greater amplitude it predominates and the resultant wave is in the reversed direction.

Up to this point we have considered the characteristic electric wave, obtained during rhythmic contraction, in its general outline. We shall now take up several details:

1. The part of the typical wave corresponding to contraction, that is, the first half of the wave, is further divisible descriptively into two elements, quite sharply defined (fig. 5). The first of these is a rapid upward deflection occupying about one-fifth of the entire contraction period, and ending abruptly. This is followed by a deflection, indicating a stationary, or slowly rising negative potential occupying the rest, that is, the last four-fifths of the contraction period. The latter is completed by a short rapid rise just before the beginning of relaxation. The relaxation phase, being the mirror-image of that corresponding to contraction, consists of two similar parts occurring in reverse order. It is to be presumed that the two component parts of each half wave of electric potential so strikingly different in appearance, represent different processes in the physiology of muscular activity. These findings raise the question of what may constitute these physiologic differences.

2. The contraction phase of the electric record contains one detailed character not present in that of relaxation. Just after the sharp rise at the beginning of contraction, there is a rapid oscillation of the string at the rate of about 10 per second. It can be seen clearly in the enlarged figure 4 d and can be made out in the waves of figure 3. These oscillations are not always observed, but are seen so frequently that we are inclined to believe that if they are absent, it is because of a lack of sufficient sensitivity in the recording devices. We have seen the same sort of rapid oscillation of the string, regularly, during tetanic contraction of the intestine, when studying peristaltic rushes. Perhaps, during rhythmic contractions, too, they represent a tetanic-like state of the muscle as a part of the contraction process.

3. The next feature to be considered is not evident in all records. In fact, it was not observed until late in our investigations, and only after special consideration did we decide that it forms a definitive part of the potential wave. We refer to the appearance, at times, of a sharp positive deflection just when one wave ends, and the next begins. This is shown in figure 4 e, in which the level of zero potential relative to the standard electrode of the bath is shown by the line at the right. When the shadow of the string is below this line, the intestine at the point of attachment of the

electrode is at a positive potential, when above, at a negative potential relative to the standard electrode. It is to be observed that at the end of a wave there is a rapid deflection of the string below the zero line (positive) and an equally rapid return merging into the next wave. The sharp downward deflection referred to is, then, due to a relatively positive potential of the intestine.

Considering the conditions of the experiment, the foregoing observation is surprising. According to the almost universally stated theories, action currents in muscle originate in a wave of negativity that accompanies excitation. According to this conception, a positive deflection in the action curve can be produced only when a bipolar record is made, and in that case the positive deflection is due to an active negativity in the distal electrode. But we were working with a unipolar arrangement. It is difficult to frame any hypothesis by which the standard electrode in the bath could be affected negatively by the contraction of the intestinal muscle far removed from it. But, more important still, it is well nigh impossible that it should be so affected, in such precise relationship to the phases of the wave of potential from the muscle, for these phases do not coincide in time for waves from different parts of the intestine. A negative pulse at the standard electrode, coördinated to the phases of one wave, would necessarily be out of phase with another. We seem, therefore, to be driven to the conclusion that the origin of the downward throw of the string referred to, is produced by a positive potential at the site of the active intestine. For such a phenomenon there is no place in the negativity hypothesis usually advanced to explain action currents from muscle and nerve. Similar observations have been made for striated muscle by Craib.

4. Another point is noteworthy because of its contrast with what is seen in the usual action-current tracings. We have shown that the wave of potential during relaxation is quite as marked in its definitive character as that during contraction. Such is not the case for action currents from striated muscle or nerve ordinarily. For instance, the entire P-Q-R-S-T wave of the electrocardiogram is completed during systole; no changes in electric potential occur during diastole. The same is essentially true for skeletal muscle; the electric changes observed during relaxation are merely due to the passing off of the previously established negative potential. This is not the case in our observations; there was a definite character to the potential wave during relaxation (the same indeed as during contraction), it has a point-to-point correspondence with the mechanical record of relaxation, and its duration in time is exactly the same. In view of the fact that it is so unusual to observe action-current changes during relaxation of muscle, we hesitate to advance this observation as contradictory of the general finding.

It is conceivable that what we have observed in these experiments is

not strictly comparable with the ordinary action currents. Also it may be that the lengthening of the muscle is not always due entirely to relaxation, but is due partly at least to a pull resulting from contraction of the muscle in neighboring segments of the intestine. It is hard to see how such stretching could produce the particular form of wave observed, but the possibility should be kept in mind. It would not be surprising, however, to find indications of active potential changes accompanying relaxation in smooth muscle because this tissue is always in a state of tonus between complete relaxation and complete contraction. Since relaxation as well as contraction can be produced by stimulation of the appropriate nerve it may be looked on perhaps as an active process at least under some circumstances.

The wave of electric potential, which we have described for rhythmic contractions of the rabbit's intestine, does not appear to be limited to that animal. We have found evidence of its presence in some experiments on the dog and cat which we hope to report later. Puestow, working with segments of the dog's intestine transplanted to the abdominal wall obtained action currents which we would interpret as corresponding to the inverted type of wave in a "bipolar record" obtained in the rabbit. For comparison an example from Puestow's records is included in figure 3 c. Electrograms obtained by Castleton also of isolated dog's intestine, which he has kindly permitted us to examine, corroborate this impression.

SUMMARY

Simultaneous electric and mechanical records from the intact intestine of the anesthetized rabbit were made. Bipolar and unipolar records were studied. The unipolar is fundamental; the bipolar represents the algebraic difference between the unipolar potential changes at the two electrodes. During rhythmic contraction of the intestine, a characteristic type of wave of electric potential can be observed which has a definite relation to the mechanical contraction. The electric wave has a symmetric form; the first half occurs during contraction and the second half during relaxation. As a part of the contraction phase there are often a number of small rapid oscillations similar to those observed during tetanus. Unipolar records show that a positive variation sometimes occurs during the course of the contraction process.

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EFFECTS OF CORTICO-ADRENAL EXTRACT ON GLYCOLYSIS IN VITRO[1]

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Experiments recently carried out in this laboratory have shown that the adrenal cortex is intimately concerned with the regulation of carbohydrate metabolism in normal and in adrenalectomized animals (Britton and Silvette, 1931, 1932). Cortico-adrenal extracts which were made according to a slightly modified Swingle-Pfiffner technique increased hepatic and muscle glycogen and blood sugar, and concurrently decreased the blood lactates. Since the effects of the extract on carbohydrate metabolism in the intact animal were so well marked, it was thought that additional knowledge concerning the relationship of cortical extract to the metabolism of carbohydrates might successfully be acquired from experiments *in vitro*.

Earlier work on the effects of insulin and adrenalin on muscle-glucose systems has been mostly inconclusive (Cori, 1931). In the present investigation it was decided to work mainly with a blood-glucose-extract system, since the physical and chemical characteristics of blood lend themselves to accurate and easily controlled treatment.

To date we have made about 130 batches of cortico-adrenal extract (Swingle and Pfiffner, 1931), and have found the material effective on adrenalectomized and normal animals. The different extracts were not equally potent, however, so that experiments reported herein cannot always be quantitatively compared one with another unless the same extract happened to be employed in each case.

METHODS. Blood was withdrawn by syringe and needle from the hearts of normal, well-nourished dogs, and defibrinated by shaking in a flask with a number of glass beads and filtering through a wisp of cotton. Immediately after defibrination it was carefully mixed, measured out and used.

[1] Reported at the annual meeting of the American Physiological Society in Philadelphia, April 29, 1932.

[2] Porter Fellow of the American Physiological Society.

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Sufficient amounts of a solution of pure glucose in 0.8 per cent sodium chloride were added to bring the absolute amount of glucose present to between 10 and 15 mgm. (see tables). The cortico-adrenal extracts and also the 1:2,000,000 adrenalin control solution employed were made isotonic with sodium chloride before being added to the blood. Sufficient normal saline solution was then added to bring the contents of the tubes to 10 cc. The erythrocytes were thus suspended in a matrix of the same pH and osmotic pressure as that of whole blood.

Our extracts when quite fresh (immediately after having been Seitz-filtered) have been observed to have a pH slightly above 7 by the quinhydrone electrode. With age, however, the extracts seemed to grow more acid; extracts a week old commonly had a pH of 6.0 to 6.5, while still older ones have been as acid as pH 4.5 to 5.0. In these experiments fresh extracts have been used in most cases, although the following experiment showed that even relatively small quantities of defibrinated blood had sufficient buffer action to take care of relatively large amounts of an extract as acid as pH 4.52 (extract no. 122, two weeks after completion):

Tube 1: 1 cc. blood, 0.25 cc. extract, 3.75 cc. saline; pH 7.48.

Tube 2: 1 cc. blood, 1.50 cc. extract, 2.50 cc. saline; pH 7.44.

After careful mixing of the contents of the tubes, samples for glucose analysis were withdrawn by means of a 0.1 cc. Folin blood sugar pipette, care being taken that the same pipette was used throughout an experiment. The tubes containing the blood-glucose-extract mixture were placed in a specially constructed shaker-incubator maintained at a temperature of 38 to 39 degrees, in which they were shaken about 70 times a minute for a period of three hours. Intermediate and final samples for analysis were taken only after the contents of the tubes had been again cooled to room temperatures. Samples for lactic acid determinations were collected with similar precautions before and after incubation. Practically all experiments were run in pairs; furthermore all determinations were made in duplicate, i.e., the figures given in the tables represent the average of 2 to 4 readings.

Glucose was determined by the method of Folin and Malmros (1929), and lactic acid by the method of Friedemann, Cotonio and Shaffer (1927).

Results. In order to determine the normal variation in the rate of glycolysis in a series of control tubes, a number of experiments were performed as follows:

Equal quantities of defibrinated blood from a normal animal were suspended in equal volumes of glucose-saline solution, and the tubes containing the blood-glucose mixture placed in the shaker-incubator for three hours. Initial and final blood glucose determinations were made. A typical control experiment is given below.

Forty per cent defibrinated blood, 60 per cent glucose-saline solution. Incubated 3 hours. Initial glucose concentrations, respectively, 154, 157, 155, 157, 156, 154 mgm. per cent. Final glucose concentrations, respectively, 130, 126, 126, 133, 128, 126 mgm. per cent. Per cent decrease in glucose over 3 hour period, respectively, 15.6, 19.7, 18.7, 15.3, 18.0, 18.1,—mean, 17.6.

In the above experiment, the greatest positive divergence was 11 per cent greater than the mean, and the greatest negative divergence was 13 per

TABLE 1

Effects of different amounts of cortico-adrenal extract on glycolysis in vitro

EXPERIMENT	BLOOD	EXTRACT	GLUCOSE		DECREASE IN GLUCOSE	CHANGE FROM CONTROL
			Before incubation	After incubation		
	per cent	*per cent*	*mgm. per cent*	*mgm. per cent*	*per cent*	*per cent*
18	15	0	126	117	7.1	
	15	5	120	109	9.2	+ 30
	15	10	123	98	16.3	+129
	15	20	120	115	4.2	−41
	15	30	126	121	4.0	−44
5	30	0	146	125	14.4	
	30	10	148	119	19.6	+36
10	30	0	135	120	11.1	
	30	5	139	114	18.0	+62
	30	10	139	122	12.2	+10
	30	30	142	130	8.5	−23
6	35	0	151	139	8.0	
	35	10	152	136	10.5	+31
21	60	0	144	107	25.9	
	60	2.5	142	87	38.8	+44
	60	10	142	118	20.3	−21
	60	30	142	125	12.0	−54
14	87	0	105	47	55.2	
	87	9	105	53	49.5	−10
	87	16	100	58	42.0	−24

Experiments 2, 8, 9, 11, 17, 19 and 20 resulted similarly; details omitted in the interests of conservation of space.

cent less than the mean. From table 1 it will be apparent that in no case in the extract-containing tubes was the increase in glycolysis less than 17 per cent more than that occurring in the control tubes, with the great majority of increases above the control rates over 30 per cent. Conversely,

in only one case in which the inhibitory effect of the extract is demonstrated is the decrease in the rate due to extract less than 20 per cent below the rates occurring in the control tubes.

The presence of cortico-adrenal extract within limits in the blood-glucose system induced a markedly higher rate of glycolysis than was observed in the control tubes (table 1). Large quantities of the extract (20–30 per cent concentration) nevertheless inhibited glucose disappearance very considerably. In this connection it is interesting to note that Ahlgren (quoted by Cori, 1931) found that the optimal concentration of insulin

TABLE 2

Effect of adrenalin and boiled cortico-adrenal extract on glycolysis in vitro

EXPERIMENT NUMBER	BLOOD	SUBSTANCE ADDED		GLUCOSE		DECREASE IN GLUCOSE	CHANGE FROM CONTROL
		Per cent	Material	Before incubation	After incubation		
	per cent			*mgm. per cent*	*mgm. per cent*	*per cent*	*per cent*
5-A	30	10	Saline	146	125	14.4	
	30	10	Adrenalin*	142	120	15.4	+7
10-A	30	10	Saline	100	85	15.0	
	30	10	Boiled extract	101	86	14.8	−1
3-A	35	10	Saline	168	144	14.3	
	35	10	Adrenalin*	167	140	16.2	+13
6-A	35	10	Saline	151	139	8.0	
	35	10	Adrenalin*	137	129	5.9	−25
8-A	35	10	Saline	108	93	13.9	
	35	10	Adrenalin*	107	89	16.8	+18
11-A	50	5	Saline	95	76	20.0	
	50	5	Adrenalin*	94	75	20.2	+1
	50	5	Boiled extract	94	75	20.2	+1
14-A	87	9	Saline	105	47	55.2	
	87	9	Adrenalin*	105	50	52.3	−5
	87	9	Boiled extract	105	54	48.5	−12

* Per cent of a solution containing 1 part of adrenalin in 2 million.
Tubes shaken 70 times per minute at 38°C., for 3 hours.

necessary to reduce the decoloration time of methylene blue by frog muscle *in vacuo* was 10^{-9} to 10^{-15}; at 10^{-5} insulin showed an inhibitory effect.

An interesting relationship was found to occur in the blood-glucose-extract system. For a given concentration of blood there is an optimal concentration of extract necessary to give the maximal rate of glycolysis. Extract concentrations lower than the optimal do not give maximal decreases in glucose, while extract concentrations higher than the optimal inhibit the glycolytic reaction (table 1). A change in the concentration of blood shifted furthermore the optimal extract concentration. In general

it was observed that the greater the percentage of blood, the smaller the percentage of extract necessary to give maximal glycolysis. The reverse condition also held true: the smaller concentrations of blood required larger amounts of extract to give maximal effects.

In several experiments the lactic acid concentrations were determined before and after incubation. There was no significant change in the levels, however, nor any differences between the control and extract-containing tubes, and this line of investigation was not further pursued.

Cortical extract which had been boiled for two minutes in an open flask was found to be ineffective in increasing the control rates of glycolysis. Adrenalin solution (containing adrenalin in similar concentration—1:2,000,000—to that found in our extracts), used in control tubes in amounts equal to the quantities of extract used in the principal experiments, was also without effect on the (control) rate and amount of glucose decrease (table 2).

In one experiment in which the extract was added to blood-glucose in the presence of 0.002 M sodium cyanide the rate of glycolysis was found to be approximately the same as that occurring in the same system without the cyanide.

Statistical analysis of the glycolytic rates observed in extract-containing and control tubes indicates the validity of the results:

Mean, control tubes (M_c): 14.1 $\pm$ 0.98 per cent glycolysis.

Mean, extract tubes (M_e): 20.6 $\pm$ 1.21 per cent glycolysis.

Difference, $M_e - M_c$: 6.5.

Probable error of this difference: $\pm$1.55.

SUMMARY

Cortico-adrenal extract increases the rate of glycolysis in the presence of normal defibrinated blood *in vitro* from 25 to 125 per cent (average 50 per cent) above that found in the control tubes.

The increased glucose utilization is a function of the relationship between the concentrations of blood and cortical extract: small amounts of blood require relatively large amounts of extract, and *vice versa*, in order to attain the maximal rate of glycolysis. Amounts of the extract greater than the optimal inhibit glycolysis.

Extracts which have been boiled in an open flask do not increase the rate of glucose disappearance.

Adrenalin used in dilution equal to that found in cortical extracts (1:2,000,000) is also ineffective.

Our cortico-adrenal extract changes from a pH of about 7.0 when freshly made to pH 4.5–5.0 when several weeks old. This is probably due to acid decomposition products of the small amount of adrenalin contained in the material.

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BLOOD-CELLULAR CHANGES IN ADRENAL INSUFFICIENCY AND THE EFFECTS OF CORTICO-ADRENAL EXTRACT[1]

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The muscular incapacity, the circulatory disturbance and the disordered carbohydrate metabolism observed in adrenal insufficiency in animals and in man have been referred to in earlier reports (Britton, 1930; Britton and Silvette, 1931, 1932). The profound deficiencies which occur in the metabolism of carbohydrates have in our experiments appeared to be the dominant feature of adrenalectomy; the alleviatory effects of extract of the adrenal cortex appear furthermore to be directed mainly toward restoration of carbohydrate balance. That there are other important cortico-adrenal functions is none-the-less admitted. In a later report (Eagle, Britton and Kline, 1932) the influence of cortico-adrenal extract on muscular activity has been considered. Evidence on the extensive blood-cell changes in adrenal insufficiency, and the notable effects of extract administration, is now briefly presented.

The marked increase in blood concentration which follows adrenalectomy and the recovery to normal which is effected by cortico-adrenal extract have previously been reported (Britton and Silvette, 1931). These and other observations suggested the desirability of a more intimate study of probable variations in the blood-cellular elements under similar experimental conditions. Determinations have been made of the erythrocyte and leucocyte counts in cats under *a*, normal conditions; *b*, after adrenalectomy, and *c*, after extract treatment. Total and differential leucocyte determinations were carried out. Studies were further made on a number of rats and rabbits, and various control experiments were also performed. About fifty animals were studied. The adrenals were removed by a one-stage operation, the average survival period for cats being $6\frac{1}{2}$ days. Cortico-adrenal extract made by a modified Swingle-Pfiffner procedure (Britton and Silvette, 1931) was used.

Erythrocytes. Red blood cell determinations were made in ten experiments on cats. The normal pre-operative counts for this group averaged 9,800,000 red cells per cu. mm. With the development of adrenal insuffi-

[1] Grateful acknowledgment is made of aid received in this investigation from the Grants-in-Aid Committee of the National Research Council.

ciency the number always rose considerably. The average count in the case of animals showing more or less severe symptoms was 15,500,000—an elevation of about 60 per cent above normal. This increase is in keeping with the reported observations on the changes in blood cell volume which follow removal of the adrenal glands (Britton and Silvette, 1931).

Cortico-adrenal extract was observed to bring about a notable reduction in the erythrocyte count in the course of a few hours, in some cases to approximately the pre-operative value. Adrenalin used in the concentration usually present in our cortical extract (1:2,000,000), and given in a large amount of fluid, failed to influence the cell values, however, to any significant degree. The protocol given below is illustrative. It will be noted that an increase in the red blood cell count began shortly after operation; while just before death, which supervened after extract had been stopped, the erythrocytes had almost doubled in number.

Cat 99; weight 2.2 kilos.

June 14, 9:00 a.m., normal r.b.c. 8.6 million; animal adrenalectomized
June 15, 9:00 a.m., r.b.c. 8.6 million
June 16, 9:00 a.m., r.b.c. 12.1 million, cat weak
12:30 p.m., r.b.c. 14.6 million, very weak
12:55 p.m., given 25 cc. adrenalin (1:2,000,000 intraperitoneally)
3:00 p.m., r.b.c. 15.7 million, animal very weak, unable to stand
3:15 p.m., 30 cc. cortico-adrenal extract (intraperitoneally)
5:00 p.m., r.b.c. 14.3 million
10:00 p.m., r.b.c., 9.8 million, marked improvement, cat walking about
June 17, 9:00 a.m., r.b.c. 12.3 million; no injections
June 18, 9:00 a.m., r.b.c. 14.5 million; no further extract given
June 19, 9:00 a.m., r.b.c. 17.0 million. Cat died on this date

The data given in table 1 further show the changes produced by cortico-adrenal extract, in comparison with the negative results of adrenalin and saline injections. Glucose solution was also ineffective. The abnormally high erythrocyte levels which follow adrenalectomy are strikingly reduced by extract administration during the time that general recovery of the animal is brought about. It would seem possible and likely that the reductions occur because of blood dilution, although no fluid was given to the animals other than that contained in the extract.

Leucocytes: Total counts. Noteworthy changes were observed in the leucocyte counts in cats following adrenal removal and the subsequent development of symptoms of insufficiency. Normal total and differential determinations were made before operation, and continued daily after excision of the adrenal glands.

In a few cases the total white cell counts appeared abnormally high, varying between 14,000 and 28,000 per cu. mm., before operation. The normal range in most cases appeared to be between 7,000 and 14,000 cells.

In eight experiments on animals which were carefully followed, the leucocyte count averaged 12,400 before operation. Removal of the adrenals resulted in a reduction in these cases within a few days to an average of

TABLE 1

Showing the marked increase in erythrocytic counts (given in millions per cu. mm.) after adrenalectomy, and the influence of various injected materials

CAT NUMBER	NORMAL ERYTHROCYTES	ERYTHROCYTES IN ADRENAL INSUFFICIENCY	MATERIAL INJECTED	ERYTHROCYTES AFTER TREATMENT	DIFFERENCE AFTER TREATMENT	PERCENTAGE DIFFERENCE
95	11.2	18.6	Adrenal extract	13.9	−4.7	−25
97	6.9	17.6	Adrenal extract	13.3	−4.3	−24
99	8.6	15.7	Adrenal extract	9.8	−5.9	−38
92	11.9	14.1	Adrenalin (1:2,000,000)	14.2	+0.1	+1
97	6.9	17.3	Adrenalin (1:2,000,000)	17.1	−0.2	−1
99	8.6	14.6	Adrenalin (1:2,000,000)	15.7	+1.1	+7
93	11.3	14.0	Saline (0.9 per cent)	13.3	−0.7	−5
96	9.4	13.8	Saline (0.9 per cent)	13.6	−0.2	−1
100	8.9	14.7	Saline (0.9 per cent)	17.3	+2.6	+18

Cortico-adrenal extract and control solutions were given by intraperitoneal injection in dosage of 10 cc. per kilo.

TABLE 2

Changes in total leucocytic counts after adrenalectomy and the influence of cortico-adrenal extract and of adrenalin (controls)

CAT NUMBER	TOTAL LEUCOCYTES (NORMAL)	LEUCOCYTES BEFORE INJECTION (AFTER OPERATION)	MATERIAL INJECTED	LEUCOCYTES AFTER INJECTION	DIFFERENCE AFTER INJECTION	AVERAGE DIFFERENCE
71	10,400	7,600	Adrenal extract	11,200	+3,600	
71		10,800	Adrenal extract	16,800	+6,000	
71		11,200	Adrenal extract	24,200	+13,000	+6,500
76	13,600	8,800	Adrenal extract	12,400	+3,600	
76		12,400	Adrenal extract	18,600	+6,200	
56	20,800	10,400	Adrenalin	5,600	−4,800	
58	7,800	5,800	Adrenalin	4,000	−1,800	−2,900
63	10,800	9,200	Adrenalin	6,800	−2,400	
64	10,000	5,600	Adrenalin	2,800	−2,800	

7,000 cells. In one animal there was a fall in the total count to 2,800 and in another to 4,000 cells before death.

The effects of administering cortico-adrenal extract to animals which were showing symptoms of adrenal insufficiency were invariably striking. Associated with the process of recovery, the total white counts were con-

siderably augmented—in some cases to relatively high limits. Cat 71 showed an increase, for example, from 7,600 to 24,200 cells after three injections of extract (table 2). In control experiments in which adrenalin was injected no such responses were observed; the leucocyte counts continued to fall, indeed, until the animals succumbed.

Differential leucocyte counts. Adrenalectomy resulted in very significant changes in the differential leucocyte counts. Within a day or two after operation, the neutrophiles were found to be reduced, and the lymphocytes correlatively increased in percentage. These changes were progressive until wide variations from the normal levels were observed with the development of symptoms of insufficiency. In some cases the neutrophiles

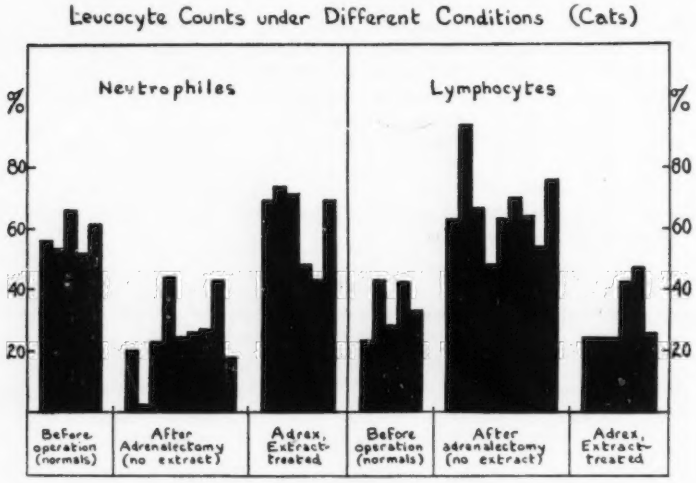

Fig. 1

almost entirely disappeared; they were reduced to the vanishing level of 3 per cent, with the appearance of adrenal insufficiency, in the case of animal 46.

The administration of cortico-adrenal extract brought about a complete reversal of the disorganized blood-cellular picture observed in adrenal insufficiency. Several hours were often necessary to effect the complete change; it took place coincidently, however, with the restoration of the animal to normal activity. The changes are shown in the experiment outlined in table 3, and in summary in table 4.

Shifts in the differential cell count toward the normal were sometimes brought about by adrenalin administration. These changes were very slight, however, and always short-lived.

TABLE 3

Experiment: Cat 46: Showing the effects on the differential leucocyte counts of adrenalectomy and of the administration of cortico-adrenal extract

DATE AND TIME	CONDITIONS	MYELOCYTES	NEUTROPHILS	EOSINOPHILS	BASOPHILS	LYMPHOCYTES	MONONUCLEARS
		per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
Mar. 12	Normal	0	53	2	1	43	1
Mar. 12	Adrex.*						
Mar. 21		1	12	5	1	74	7
Mar. 22		0	3	0	0	94	3
Mar. 22	Extract given						
5 p.m.							
6 p.m.		0	16	3	1	74	6
11.30		1	24	1	0	63	11
Mar. 23	Extract given	0	23	1	0	67	9
9 a.m.							
11 a.m.		0	39	1	0	53	7
12 m.		0	60	2	1	29	8
9.30 p.m.		0	69	1	0	24	6

* Bilateral adrenalectomy.

TABLE 4

Showing the fall in neutrophils and the increase in lymphocytes after adrenalectomy, and the restitutional effects of administering cortico-adrenal extract

(Adrenalin was ineffective)

CAT NUMBER	NORMAL		BEFORE EXTRACT (ADRENAL INSUFFICIENCY)		AFTER EXTRACT	
	Neutrophils	Lymphocytes	Neutrophils	Lymphocytes	Neutrophils	Lymphocytes
	per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
46	53	43	3	94	24	63
46			23	67	69	24
47	66	28	44	47	73	24
47			24	64	70	24
49	52	42	26	70	48	43
49			27	64	43	47
51	61	33	43	54	69	25
51			18	76	32	62
76	80	14	62	36	91	8
Average. . . .	63	32	30	64	58	36

Positive effects of adrenal extract on the differential white counts were observed furthermore in normal and adrenalectomized white rats and in normal rabbits, as well as in operated cats. In adrenalectomized rats adrenalin appeared to be able to raise the (previously reduced) neutro-

phile counts, although not as markedly as cortico-adrenal extract. The difference in reaction to adrenalin between the rat and the cat may possibly be due to the wide difference in the normal neutrophilic content of the blood of these animals. The normal rat possesses approximately one-half the percentage of neutrophiles present in normal cat blood. Comparison of the differential effects of various substances on the blood of these forms may thus be impossible. Accessory cortico-adrenal tissue may also complicate the conditions in the case of (apparently) adrenalectomized rats.

Splenectomy. In several control cats the spleen was removed and the blood thereafter studied for possible changes which might be attributed to the absence of this organ. No significant variations from the normal leucocyte levels were observed, although subsequent adrenalectomy quickly brought about changes similar to those noted above. In these splenecto-adrenalectomized animals cortico-adrenal extract was effective in restoring the normal leucocyte levels, while control experiments with adrenalin were negative.

Discussion. It is interesting to note that Addison in his classical monograph on adrenal disease (1855) indicated that the circulatory manifestations of the condition were of considerable importance. Anemic and other features of the disease led him to the belief, indeed, that the adrenal glands were "directly or indirectly concerned in sanguification." The present data give support to this very early prediction.

Extensive changes in the leucocytic content of the blood of adrenalectomized cats have been observed by Zwemer and Lyons (1928). Removal of the adrenals in their cases was followed, in the longer-surviving animals, by a decrease in the percentage of polymorphonuclear neutrophiles and an increase in the percentage of lymphocytes. The total number of leucocytes was found to be decreased, as a rule, in adrenal insufficiency. Stewart (1926) and Rogoff and Stewart (1926) have called attention to the marked decrease in the plasma content of the blood following adrenalectomy in the dog. These workers further noted considerable increases in the erythrocyte counts, and also in the hemoglobin percentage and specific gravity of the blood. Observations made on the cat in this laboratory (Britton and Silvette, 1931) are in agreement with these findings.

It seems likely that the changes in red blood cell concentration which occur in adrenal insufficiency are indications of the extensive shifts in water balance in the organism. The profound effects of the operation on the leucocytic levels probably represent blood-cellular shifts which are more specifically related, however, to the adrenalectomized animal. The results indicate the possibility, indeed, of a neutrophilogenic failure in the adrenalless organism, with an apparent related lymphocytosis. The possible involvement of the bone marrow and other tissues in adrenal insufficiency is thus indicated, and this phase of the problem is now being investigated.

It may be worth while to consider whether the condition of neutrophilopenia which supervenes in adrenalectomy has any relationship to the condition of "agranulocytosis" as observed in man. The individual's "chief cellular defense mechanism, the neutrophilic leucocyte" (Doan, 1932) is profoundly disturbed in both cases. The evidence now emphasizes the prompt relief afforded by treatment of the experimental animal with cortico-adrenal extract, although the specificity of the reaction is not at present considered. General factors, possibly those involving tissue permeability, may nevertheless be concerned.

It seems reasonable to suppose that such a highly differentiated (and probably relatively unstable) tissue as the adrenal cortex may be disorganized oftener and more readily than suspected, and such disorganization may indeed be implicated in neutropenic conditions observed in man. The pathological effects of adrenal removal are widespread and profound, and it would appear likely that many diseases of now unknown etiology may be explicable on the basis of cortico-adrenal hypofunction or dysfunction. The increasing availability and knowledge of extracts of the adrenal cortex surely suggest that more extensive tests of the therapeutic value of the material be now undertaken.

SUMMARY

Adrenalectomy produces marked numerical changes in the cellular elements of the blood. With the development of symptoms of adrenal insufficiency, the erythrocytes increase commonly from 50 to 100 per cent; this change is probably due to fluid loss from the blood. The total leucocyte counts are meanwhile found to be decreased to a similar extent. There are pronounced reductions in the neutrophile counts, sometimes almost to the disappearing point. The lymphocytes show a concomitant increase in percentage.

The administration of cortico-adrenal extract to animals suffering from severe adrenal insufficiency and showing the above profound blood-cellular disorganization resulted in complete restitution of the normal cell values. Recovery of the blood cell elements to normal values was coincident with general improvement in the condition of the animal.

Control experiments with adrenalin, glucose and saline solutions were negative, with the exception of the partly effective action of adrenalin on the white rat.

There were no noteworthy leucocytic changes in splenectomized controls. Splenecto-adrenalectomized animals showed responses after operation and after extract treatment, however, which were similar to those noted above.

The possibility that the neutrophilopenia of adrenal insufficiency is related to the clinical condition of "agranulocytosis" is considered.

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THE INFLUENCE OF CORTICO-ADRENAL EXTRACT ON ENERGY OUTPUT[1]

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In earlier papers we have presented brief reports which indicate a significant influence of cortico-adrenal extract on the ability of dogs to perform muscular work (Britton and Silvette, 1931; Eagle and Britton, 1932). Continued improvement in methods of preparing the extract has now made available a material which is sterile, protein-free and contains practically no adrenalin, and which possesses significant effects on many bodily activities. We have thus been able recently to complete a series of observations begun about two years ago on the energy output of normal dogs before and after injection of adrenal extract of known potency, made according to a modified Swingle-Pfiffner method (Britton and Silvette, 1931).

The procedures for determining the working capacity of animals were similar to those used by Campos, Cannon, Lundin and Walker (1929), with whom one of us was earlier associated. These investigators noted the effects of adrenalin, glucose, insulin and various operations on the maximal running ability. In our experiments, dogs were first trained over a period of several weeks to run in a treadmill. The apparatus consisted of a cage enclosing an endless belt which was moved by means of an electric motor; through a rheostat the travelling speed of the belt could be varied from 110 to 224 meters per minute. The number of revolutions of the measured belt, inclined at an angle of 10 degrees, was calculated from the recorded revolutions of one of the pulleys: 1000 revolutions of the belt corresponded to a counter reading of 3948.

The animals, which had been kept fasting before an experiment for a period of 18 to 20 hours, were first allowed to become quiet before pulse readings and blood samples were taken. Blood was taken from the peripheral ear vessels, and the sugar was determined according to the method

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of Folin and Malmros (1929). After the "starting" pulse readings and blood samples were obtained, the dogs were allowed to run in the treadmill until, with four stops being made for observation as hereafter noted, they eventually became exhausted. The end-point used was that described by Campos, Cannon *et al.*: it was indicated by the animal lying down and refusing to run further, by refusing to rise after the treadmill had been stopped, or by promptly lying down and refusing to run when the mill had been restarted. The performance of the dogs and their condition after each bout of exercise were also designated for purposes of comparison according to the method employed by the foregoing workers as follows:

Performance: V—vigorous running; S—steady running; I—irregular running; R—refusal to run.

Condition at end of run: A—active; W—weary, panting; T—tired, panting heavily; F—fatigued; E—exhausted.

In some cases it was not possible to denote the condition of the animal adequately by one letter, and two were therefore used.

Unlike the procedure employed by the Harvard investigators, we stopped the treadmill at indicated times during the experiment so that checks on blood sugar and pulse could be taken. Thus, an animal was permitted to run as long as its running behavior could be designated by "V" (vigorous); when vigorous running was marred by more than rare stops, the treadmill was stopped and readings and samples taken. After five minutes' rest, the machine was again started and the dog permitted to run until its behavior was no longer denoted by "S." The same procedure was followed for the conditions signified by the letters "I" and "R." The dog therefore ran for four periods, and received three rest periods of five minutes each. As will be shown later, under these conditions the animal ran apparently with willingness to the point of exhaustion; further, uniformity was maintained throughout the series. Comparison of the performances of an animal throughout its experimental course was readily possible.

The animals were allowed to run in the mill once every 3 to 5 days until, after the process of training had extended over several weeks, their working ability was maintained at a fairly uniform level. After this standard working capacity, representing a maximum energy output which remained constant for several successive experiments, had been definitely established, cortico-adrenal extract was administered intraperitoneally (with one exception), and determinations of running ability again made.

It was thought that careful study of a few selected animals over fairly long periods would be of greater value than the use of many over a relatively short time. Three dogs have thus been studied over periods of four, five and seven months respectively, with preliminary training periods

of several weeks in each case. Small dogs of about 7 kilos in weight were used in order to conserve the (expensive) extract, and also to avoid the necessity of attending to very long runs (of 4–8 hours), which we found could be readily carried out by larger animals. Some dogs which we tested appeared to be almost tireless runners.

In each experiment, calculation of the total expenditure of energy in excess of basal metabolism was carried out. This was possible through the use of average factors obtained from the determinations made by various investigators. Zuntz (1903) has reported that for moving 1 kilogram of a dog weighing 29.35 kgm. through a horizontal distance of 1 meter the energy expenditure in excess of basal metabolism did not vary when the running rate was 57.4 meters per minute or 101.1 meters per minute. Our rates of speed did not show nearly such a wide variation, and it can be considered that the slight changes which did occur exerted little or no influence on the results.

The average of the determinations made by Zuntz (1903) and Slowtzoff (1903) was 0.67 kgm-m.; Frentzel and Reich (1900) reported a value of 0.501; and Anderson and Lusk (1917) 0.58, with a maximum variation of ± 1.7 per cent. These figures have been derived from experiments on animals of various weights running at widely different rates. Campos, Cannon *et al.*, using dogs from 10 to 12 kgm. in weight, considered 0.6 kgm-m. as the energy expenditure to move 1 kgm. of dog 1 meter of horizontal distance. Although our dogs weighed somewhat less, it was considered that the same value (0.6 kgm.-m.) could be justifiably used. Since we are interested primarily in the percentage change of total energy output after administration of the extract, the use of a somewhat higher factor would involve no difference in the final analysis.

Considering the values for vertical work, Zuntz and Slowtzoff have noted that the energy output for elevations from 14.2 to 23.6 per cent is practically constant. The average was approximately 3 kgm-m. per kgm. of dog raised 1 meter. From the foregoing data it has thus been possible to calculate the expenditure of energy per kilogram body weight in excess of basal metabolism as follows:

$$E = (0.6 \times a \times \cos 10°) + (3 \times a \times \sin 10°) = a \times 1.11$$

where a equals the distance run at an elevation of 10 degrees of the treadmill platform. Although, as has been pointed out by the Harvard workers, "this value is probably minimal and approximately represents the work of transporting the body, but does not include that involved in the functioning of the respiratory muscles," it amply serves the purpose of our experiments in which a percentage change in energy output is the condition to be noted.

Our earlier observations carried out on dog "Brownie" are given in brief

in table 1. After preliminary training experiments extending over about four weeks a standard working capacity had been attained. Thus, an energy output of approximately 12,000 kgm-m. represented the normal for this small 7-kilo animal. After administration of cortico-adrenal extract,

TABLE 1

Data of experiments on dog "Brownie" (male, 7 kilos), showing normal running capacity and effects of injection of cortico-adrenal extract

(See text for injection times)

DATE AND INJECTION	RUNNING TIME	RUNNING RATE	ENERGY* IN 1000 KGM-M.	BLOOD SUGAR	
				Start of run	End of run
	minutes	*m. per minute*		*mgm. per 100 cc.*	*mgm. per 100 cc.*
1.23.31	101	110	12.3	—	—
1.30.31	94	116	12.1	—	—
2. 5.31 (*10 cc. Ext.*)†	—	—	—	—	—
2.11.31 (*20 cc. Ext.*)	97	119	12.8	84	96
2.21.31	171	121	23.0	131	74
2.25.31	137	121	18.4	94	64
2.28.31	102	118	13.3	94	63
3. 2.31 (*20 cc. Ext.*)	103	121	13.8	91	89
3. 5.31	110	122	14.8	88	83
3. 7.31	138	125	19.2	92	74
3.12.31	190	124	26.2	88	74
3.15.31	147	127	20.7	85	83
3.18.31	139	129	19.9	75	64
3.22.31	132	129	18.9	99	96
4. 4.31	116	125	16.1	91	73
4. 8.31	120	122	16.2	92	85
4.15.31	117	122	15.8	95	80
4.23.31	97	125	13.5	84	75
4.30.31 (*20 cc. Ext.*)	91	124	12.5	83	74
5. 5.31	155	122	21.0	80	73
5. 9.31	96	121	12.9	89	85
5.12.31	97	120	13.0	88	79
5.20.31	95	119	12.5	87	76

* Energy calculated in 1000 kilogram-meters in excess of basal metabolism (see text).

† Intravenous injection; all others intraperitoneal.

the working capacity increased to a figure almost twice as high, to fall again within two weeks or so to the basal working level. Injections were given on the date immediately above the amount noted in each case, and just before placing the animal in the treadmill except in the experiments

on "Snookie." Two further tests showed similar results—considerable increments in energy output after extract treatment, with subsequent diminutions to the normal level. The running time, it may be observed, was increased about 80 per cent, and the distance run to approximately the

TABLE 2

Experiments carried out on dogs "Lady" and "Snookie," showing effects of treatment with cortico-adrenal extract

	DATE AND INJECTION	RUNNING TIME	RUNNING RATE	ENERGY IN 1000 KGM-M.	BLOOD SUGAR	
					Start of run	End of run
		minutes	*m. per minute*		*mgm. per 100 cc.*	*mgm. per 100 cc.*
Dog, "Lady"	4.14.32	52	120	6.9	99	102
	4.18.32	72	98	7.9	111	105
	4.21.32 (*25 cc. Ext.*)	71	105	8.3	109	98
	4.25.32	142	102	16.1	114	101
	4.28.32	112	96	11.9	96	89
	5. 2.32	99	105	10.5	104	98
	5.31.32	72	99	7.9	83	87
	6. 4.32	77	95	8.1	90	84
Dog, "Snookie"	5.17.32	89	82	8.1	74	69
	5.24.32	101	85	9.5	79	74
	5.27.32 (*20 cc. Ext.*)	92	77	7.9	84	85
	5.31.32	181	80	16.1	83	65
	6. 4.32*					
	6. 7.32	95	79	8.3	89	72
	6.17.32	96	77	8.2	92	78
	6.21.32 (*35 cc. Ext.*)	97	87	9.3	88	83
	6.24.32	183	89	18.1	112	86
	6.28.32	137	77	11.7	92	88
	7. 1.32	110	78	9.5	89	82
	7. 5.32	98	82	8.9	84	83
	7. 8.32	92	80	8.2	84	80
	7.12.32	90	78	7.8	86	80

* Dog developed fits of sneezing and run was stopped.

same extent. The rate of running was kept within a small range, thus practically eliminating the possibility of error in determinations.

The blood sugar almost invariably showed a fall at the end of exercise, in comparison with the initial (fasting) level.

Experiments which we carried out a year later than the foregoing on two other dogs and with improved adrenal extracts resulted similarly. By

reference to tables 2 and 3 it may be observed that the working capacity was doubled after extract administration. In the experiments on "Snookie" the injections were given at 9 a.m., and the dog was run at 3 p.m. on the same day. Increments in energy output in these cases appeared from 6 to 9 hours after extract was given, it will be noted. An animal exercised immediately after an injection, however, did not usually show a notable increase in running capacity on the same day.

The percentage increases in energy output after extract injection over the average of the two pre-extract runs were as follows: "Brownie"—92 per cent, 94 per cent and 62 per cent; "Lady"—100 per cent; "Snookie"—85 per cent and 108 per cent. The average increase in the experimental series was 90 per cent.

TABLE 3

Data on running time and distance run in different experiments before and after extract injection

DOG	RUNNING TIME			DISTANCE RUN		
	Before extract	After extract	Increase	Before extract	After extract	Increase
	minutes	*minutes*	*per cent*	*kgm.-m.*	*kgm.-m.*	*per cent*
"Brownie"	94	171	82	10.6	20.3	92
	103	190	85	12.5	23.6	93
	91	155	70	11.2	18.9	69
"Lady"	71	142	100	7.4	14.5	96
"Snookie"	92	181	97	7.1	14.5	104
	97	183	89	8.4	16.3	94

There appears little doubt that the adrenal glands are intimately and possibly specifically related to neuromuscular activities. In disease of the organs in man (*morbus Addisonii*), chronic and progressive asthenia is said to be the outstanding clinical characteristic observed. It has been shown, however, that remarkable recoveries may be brought about by administration of extract of the adrenal cortex (Rowntree *et al.*, 1931). Animals from which the adrenals have been removed are able to carry out only a small fraction of the work performed by normal individuals (Britton, 1930). Decline in the muscular ability of adrenalectomized animals sets in, according to our experience, very shortly after the operation, although survival may continue for ten days or more. Even the severe prostration of adrenal insufficiency may nevertheless be overcome by large doses of cortico-adrenal extract (Swingle and Pfiffner, 1931; Britton and Silvette, 1931). Metabolic studies are in keeping with these observations, and again are indicative of an adreno-neuromuscular relationship.

SUMMARY

The influence of cortico-adrenal extract on the energy output of dogs running in a treadmill has been studied. Three animals were observed for periods of four, five and seven months. After the standard running capacity had been established, the effects of adrenal extract were tested. Intraperitoneal injection of the extract was found to augment markedly the energy output up to 100 per cent or more above the normal. The average increase in six series of experiments was 90 per cent.

The running time and the distance run were also increased about 90 per cent. In one instance an animal which regularly ran a distance of about 4 miles before treatment covered over 9 miles under the influence of cortico-adrenal extract. The effects were noticeable for ten to fifteen days after injection.

Blood sugar almost invariably fell during prolonged running and its course was not notably altered by cortico-adrenal extract.

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STUDY ON THE METABOLISM OF GLUTATHIONE

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It has long been known that protein-free tissue extracts give a purple color with sodium nitroprusside and ammonia. This reaction, being characteristic for sulfhydryl groups, was considered by Arnold (1910) as due to free cysteine. Though the reduction of cystine to cysteine seems to be the first step in the catabolism of this sulfur containing constituent of proteins, there has been no experimental evidence for the general occurrence of cysteine in tissues. Using the delicate naphthoquinone sulfonic acid test devised by Sullivan (1926), Thompson and Voegtlin (1926) came to the conclusion "that tissues do not contain an appreciable amount of either cystine or cysteine." This statement must be accepted with reservation, since Hunter and Eagles (1927) isolated pure cystine from pig's liver, taking great care to exclude secondary hydrolysis of protein. Hopkins (1921) showed that the substance responsible for the nitroprusside reaction is of more complex nature. He succeeded in isolating from yeast, muscle and liver a crystalline compound, to which he gave the name glutathione. According to newer investigations (Hopkins, 1929; Kendall, MaKenzie and Mason, 1929) it is a tripeptide of glycine, glutamic acid and cysteine. The physiological importance of this substance lies in the rôle which it plays in oxidation-reduction processes of cells. Its metabolic function is dealt with in a recent paper by Hele and Pirie (1931). The relatively high concentration of glutathione in liver, testes, adrenals and lens of the eyes, and the general distribution of glutathione in tissues, led these investigators to assume that it is a metabolic end product. They studied the oxidation of active and racemic cystine, cysteine and some derivatives as well as reduced and oxidized glutathione in the animal body. The rate of oxidation of these sulfur compounds varied with the animal and the mode of administration. An average of about 70 per cent of the sulfur of cystine appeared as sulfates in the urine; for cysteine the figures are slightly lower. Glutathione, in the reduced and oxidized form, was the most completely oxidized of all the compounds studied. An average of 72 per cent of its sulfur was recovered as sulfates. Great differenences in the excretion of neutral sulfur were observed. However, no attempt to study its nature was made.

The aim of the present study was to investigate the effect of a high protein diet, and of orally and intravenously administered glutathione and its constituent amino acids on the level of blood glutathione, and to investigate the rate of oxidation of these substances in the animal body.

EXPERIMENTS. Adult bitches were used throughout this investigation. The blood glutathione was determined by the method of Mason (1930). For a convenient application see Schelling (1932). The total and inorganic sulfates in urine were determined, after the removal of phosphates, by Fiske's (1921) method. A part of the glutathione used was prepared according to the method of Pirie (1930). The remainder was a commercial brand of the Eastman Kodak Company. Judged from melting point determinations these substances were pure. The cysteine hydrochloride was obtained from the Pfanstiehl Chemical Company. Glutamic acid as well as glycine were prepared according to the methods described in *Organic Syntheses,* vol. v, page 63, and vol. iv, page 31, respectively.

TABLE 1

Effect of a high protein meal on blood glutathione of dogs

	BLOOD GLUTATHIONE			
	Dog 32, 24.5 kilos	Dog 33, 15.8 kilos	Dog 52, 20.4 kilos	Dog 55, 17.6 kilos
	mgm. per 100 cc.	*mgm. per 100 cc.*	*mgm. per 100 cc.*	*mgm. per 100 cc.*
Fasting	14.2	26.8	31.2	21.0
1st hour	13.9	24.2	33.3	22.7
2nd hour	14.1	26.1	33.8	26.9
4th hour	25.8	29.6	31.2	25.2
6th hour	21.4	20.7	29.9	17.9

A. Blood glutathione following the ingestion of a high protein meal. The dogs were kept for a preliminary period of 7 days on a diet of milk and soda crackers in amounts providing 80 calories per kilo body weight. This food was then withheld for one day and the following day 500 grams ground lean beef were given in one portion. Blood samples for the determination of glutathione were taken from the fasting animal and at intervals of 1, 2, 4 and 6 hours after the meal. The findings are shown in table 1. With one exception (dog 32) the increase in blood glutathione is slight and reaches a peak somewhere between the second and fourth hour after the ingestion of the meat.

B. Effect of orally and intravenously administered glutathione and its constituent amino acids on the blood glutathione and the excretion of total nitrogen and total and inorganic sulfates in the urine. The dogs were kept on a standard diet of milk and soda crackers; in experiment 3 a ground and dried mixture of equal parts of fresh, trimmed beef hearts and soda cracker

TABLE 2

Changes in blood glutathione, inorganic and total sulfates in urine of dogs after oral and intravenous administration of glutathione and its three constituent amino acids

DATE	TOTAL NITROGEN	INORGANIC SULFATES	TOTAL SULFATES	BLOOD GLUTATHIONE					COMMENTS
				Fasting	1st hour	2nd hour	4th hour	6th hour	
Experiment 1. Dog, 26.2 kilos. Food: 1 quart of milk, 348 grams of soda crackers									
1932	*grams*	*gram*	*gram*	*mgm. per 100 cc.*	*mgm. per 100 cc.*	*mgm. per 100 cc.*	*mgm. per 100 cc.*	*mgm. per 100 cc.*	
Jan. 28	7.68	0.362	0.373						
Jan. 30	7.63	0.368	0.381						
Jan. 31	7.75	0.361	0.374						
Feb. 1	7.93	0.448	0.466						1 gram glutathione by mouth (0.136 gram N, 0.104 gram S) 1 gram glutathione by mouth
Feb. 2	7.61	0.372	0.384						
Feb. 4	7.77	0.432							
Feb. 5	7.59	0.389	0.397						
Feb. 7	7.57	0.372							
Feb. 8	7.61	0.364							
Feb. 9	7.71	0.399							0.4 gram glutathione intravenously (0.054 gram N, 0.042 gram S)
Feb. 12	7.62	0.366	0.378						
Feb. 14	7.60	0.364							
Feb. 15	8.01	0.651							1.57 grams cysteine-HCl, 1.47 gram glutamic acid, 0.75 gram glycine p.os. (0.42 gram N, 0.32 gram S)
Feb. 16	7.62	0.366							
Experiment 2. Dog, 21.8 kilos. Food: 1 quart of milk, 213 grams of soda crackers									
Feb. 21	6.00	0.211							
Feb. 22	6.04	0.206	0.219						
Feb. 23	6.30	0.336	0.350	29.3	33.6	33.6	33.1	33.1	1 gram glutathione by mouth (0.136 gram N, 0.104 gram S)
Feb. 24	6.10	0.276	0.288						
Feb. 25	6.00	0.268	0.280						
Feb. 26	6.19	0.294	0.304	31.2	32.2	33.6	33.3	32.8	0.5 gram glutathione intravenously (0.068 gram N, 0.052 gram S)
Feb. 27	6.08	0.271	0.291						
Feb. 28	6.14	0.274							
Feb. 29	5.91	0.275	0.285						
March 1	6.10	0.387		36.5	38.7	38.2	37.9	37.1	1 gram cysteine-HCl by mouth (0.113 gram N, 0.205 gram S)
March 2	6.08	0.312							
March 3	6.10	0.269	0.279						
March 4	6.03	0.271	0.282						
March 5	6.53	0.534	0.541	34.2	33.6	33.2	33.6	34.0	1.57 grams cysteine-HCl, 1.47 gram glutamic acid, 0.75 gram glycine p.os. (0.42 gram N, 0.32 gram S)
March 6	6.32	0.292	0.299						
March 7	6.09	0.280	0.298						
March 8	6.12	0.275	0.291						

TABLE 2—*Concluded*

DATE	TOTAL NITROGEN	INORGANIC SULFATES	TOTAL SULFATES	BLOOD GLUTATHIONE					COMMENTS
				Fasting	1st hour	2nd hour	4th hour	6th hour	
Experiment 3. Dog, 20.4 kilos. Food: 400 grams beef heart-cracker mixture									
1932	*grams*	*gram*	*gram*	*mgm. per 100 cc.*	*mgm. per 100 cc.*	*mgm. per 100 cc.*	*mgm. per 100 cc.*	*mgm. per 100 cc.*	
March 10	9.76	0.396	0.402						
March 11	9.73	0.407	0.425						
March 12	9.67	0.389	0.405						1 gram glutathione by mouth (0.136 gram N, 0.104 gram S)
March 14	9.80	0.484	0.499						
March 15	9.71	0.410	0.425						
March 16	9.76	0.401	0.416						
March 17	9.66	0.450	0.459	27.3	28.7	29.4	29.0	28.5	0.5 gram glutathione intravenously (0.068 gram N, 0.052 gram S)
March 18	9.58	0.404	0.417						
March 19	9.62	0.405	0.416						
March 20	9.59	0.405	0.418						
March 21	10.34	0.697	0.708	24.5	28.7	29.4	29.6	27.8	1.57 gram cysteine-HCl by mouth (0.14 gram N, 0.32 gram S)
March 22	9.61	0.430	0.441						
March 23	10.87	0.676	0.694	29.2	30.7	31.0	30.9	31.2	1.57 gram cysteine-HCl, 1.47 gram glutamic acid, 0.75 gram glycine p.os (0.42 gram N, 0.32 gram S)
March 24	9.82	0.418	0.431						

meal was used. When sulfur and nitrogen excretion became constant the administration of the substance under investigation was started. For the oral administration the glutathione and the amino acid mixture were added to a separate small portion of the standard diet. This mixture was usually consumed in a few minutes; the remaining bulk of the diet was then fed. For the intravenous administration the substances were dissolved in the necessary amount of distilled water and adjusted to pH approximately 6.0 by tenth normal sodium hydroxide. The 24 hour urine periods were always terminated by catheterizing and washing the bladder. The results obtained in three dogs are summarized in table 2. From this it is seen that orally and intravenously administered glutathione, as well as a mixture of its three constituent amino acids, only slightly increased the blood glutathione. The increase in sulfate excretion over the average of the control days indicates that from 67 to 86 per cent of the sulfur of glutathione given per os is excreted as inorganic sulfate within a 48 hour period. Figures of about the same magnitude were obtained on intravenous administration; a top figure of 92 per cent was reached in experiment 3.

Discussion. The slight rise in blood glutathione following administration of cysteine, glycine and glutamic acid may, of course, be due to un-

changed cysteine which gives the same color reaction as glutathione. It has been repeatedly shown by different investigators that the sulfur of orally administered cystine and cysteine undergoes oxidation in the animal body and is excreted by the kidneys primarily as inorganic sulfate. The degree of oxidation and excretion varies somewhat with the species of animal and the composition of the food, especially in regard to the nitrogen-sulfur ratio.

It was *a priori* to be expected that glutathione given per os would undergo the usual catabolic changes of peptides in the gastro-intestinal tract, i.e., splitting into amino acids. The relatively slight increase in blood glutathione after oral administration of glutathione suggests that only a small part of the compound passes through the intestinal wall unchanged, or that any portion absorbed is stored in the liver which, according to Thompson and Voegtlin (1926), is one of the organs richest in glutathione. On the other hand the general occurrence of glutathione in tissues and blood leads to the assumption that it is synthesized where a physiological need arises. The factors governing this synthesis in relation to requirements are not yet known, and further investigation in this direction will be awaited with interest.

In conclusion the results may be summarized as follows:

1. A high protein meal does not materially increase the blood glutathione in dogs during the digestion period.

2. Reduced glutathione, orally or intravenously administered in amounts of 0.5 to 1 gram, increases the blood level of glutathione only 2 to 4 mgm. per 100 cc. The sulfur of glutathione is oxidized to the extent of 70 to 90 per cent and appears in the urine as inorganic sulfate within the 48 hour period. The fate of the sulphur of glutathione is, similarly to that of cysteine, administered with equimolecular parts of glycine and glutamic acid under the same dietary conditions.

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